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Bacterial Lactate Dehydrogenases

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INTRODUCTION

Lactate is an important end product of bacterial fermentation of glucose and other carbohydrates. The name of the acid is derived from the Latin word for milk (lac) because when raw milk is soured naturally, the lactose used by the bacteria is largely converted into lactate. Many species of bacteria form lactate, but the proportion of carbohydrate used which is degraded to lactate depends on the carbohydrate, on the conditions of growth, and on the metabolic pathways used. The various pathways known are outlined elsewhere (11, 84).

Many species of bacteria form some lactate, and fermentations of a mixed type are frequent. *Escherichia coli* may form 1.0 to 0.8 mol of lactate from 1 mol of glucose (11), and *Staphylococcus aureus* forms mainly lactate anaerobically but acetate aerobically (7). The main genera of bacteria which form lactate are often called collectively the lactic acid bacteria and comprise (i) the genera *Streptococcus* and *Pediococcus* and some species of the genus *Lactobacillus*, all of which convert at least 85% of the glucose used to lactate (homofermentative bacteria) (7), and (ii) the genus *Leuconostoc* and the remaining species of the genus *Lactobacillus*, which form only 1 mol of lactate from each 1 mol of glucose used; CO_2, acetate, and/or ethanol account for the remaining one-half of each glucose molecule (heterofermentative bacteria).

Homofermentative bacteria use the Embden-Meyerhof pathway and in the final step convert pyruvate to lactate and regenerate nicotinamide

adenine dinucleotide (NAD) from reduced nicotinamide adenine dinucleotide (NADH), which is formed at an earlier stage. In the heterofermentative bacteria, the conversion of pyruvate to lactate is also the major step for the regeneration of NAD. All of the lactic acid bacteria, therefore, have a large amount of NAD-linked lactate dehydrogenase (nLDH). These enzymes are cytoplasmic. Pyruvate is converted into L(+)- or D(−)-lactate by different enzymes (EC 1.1.1.27 and EC 1.1.1.28, respectively). The L(+)-nLDH's are known to be different in different genera and species and even in different strains of the same species, and the same is true of the D(−)-nLDH's.

nLDH's are found in bacteria other than the lactic acid bacteria but are probably of less importance to survival in species which are not restricted to the use of carbohydrates for growth and energy, e.g., the enterobacteria and the staphylococci (Table 1).

Another type of LDH, which does not use NAD/NADH as coenzyme, is found in a variety of bacteria (Table 1). Such enzymes are known as NAD-independent LDHs (iLDH's) (EC 1.1.99. −) and have different functions in different species. They convert lactate to pyruvate, and there is little evidence of the reverse reaction occurring in vivo or in vitro. In many cases the natural hydrogen acceptor is not known.

In the lactic acid bacteria the iLDH's are cytoplasmic, but in other types of bacteria they are frequently associated with particles or are in the membranes of the cells (see below).

Lactic acid bacteria live in many different habitats where they grow and multiply actively only when most of the large amount of pyruvate formed from glycolysis is converted to lactate. They have other energetically less efficient ways of using pyruvate, but these are utilized only when the nLDH is suppressed, and then growth is slow. nLDH must be regarded as a key enzyme in energy production for this large and important group of bacteria. An understanding of the characteristics of the nLDH and of its function and regulation in many different species and genera will assist in understanding the effect which the lactic acid bacteria have on their environment and on the other organisms living in association with them.

Some lactic acid bacteria are found in the guts of animals, where their activities affect the well-being of the hosts; others are associated with dental caries, and a few are pathogenic. The majority, however, live free of any host species, are nonpathogenic, and produce no products toxic or unpleasant to humans. Therefore, humans can use edible material preserved by the growth of lactic acid bacteria; reducing conditions and low pH inhibit the growth of other bacteria which would render the food unpalatable. The history of such foods (fermented milk, butter, cheese, etc.) is long, and although in the past the preparation of these foods was traditional and not understood, today they are made by using specially selected strains and controlled conditions. It is important to know how to control fermentation so that lactic acid, and not less desirable end products, is formed. In other foods, such as wine, the lactic acid bacteria are also important. Here, metabolism may be diverted away from glycolysis, but an understanding of the growth of bacteria in acidic fruit juices still requires an understanding of the functioning of their nLDH's. Another important role of lactic acid bacteria involves animal food. Traditionally, grass was preserved for animal food as hay, but now more of the nutrients are kept when herbage is converted to silage. This process became possible only when fermentations by lactic acid bacteria were understood.

In recent years it has become possible to clarify the relationships among organisms through comparative studies of their proteins. Evolutionary trends are becoming clearer, but much information is still needed. LDHs are common not only in bacteria but also in animals. A study of these proteins should yield information which would be useful in working out the relationships among various procaryotes and between procaryotes and eucaryotes. There is much variation in the conditions under which the bacterial LDHs function, but it is not yet known to what extent this is a reflection of differences in enzyme protein.

At this stage knowledge is fragmentary, and work has been haphazard. Therefore, in this review I have tried to collect what is known of the functioning and structure of bacterial LDHs. I also show how this information has been used so far in bacterial taxonomy.

TYPES OF BACTERIAL LACTATE DEHYDROGENASES (LDHs)

Table 1 shows the types of LDHs which have been found in bacteria and the species in which these enzymes have been examined. Lactate racemases (EC 5.1.2.1) are also included, as they have been sought in strains which form DL-lactate, particularly when there has been no clear evidence of separate LDHs forming D(−)- and L(+)-lactate.

Nicotinamide Adenine Dinucleotide-Linked LDHs (nLDH's)

All nLDH's form lactate from pyruvate, and there is no evidence that they function in the other direction in vivo. Bacteria which form

TABLE 1. *References about nLDH, iLDH, and bacterial lactate racemases*

Taxon	Reference(s)					
	nLDH			iLDH		Lactate racemase
	Forming D(−)-lactate	Forming L(+)-lactate	FDP-activated, forming L(+)-lactate	Forming D(−)-lactate	Forming L(+)-lactate	
Lactobacillus						
L. acidophilus	34–36	34–36, 40, 45		34	34[a]	
L. bulgaricus	34, 36					
L. delbrueckii	34,36			36		
L. jensenii	34–37				36[a]	
L. jugurt-helveticus	34, 36	34, 36		34		
L. lactis	28, 34, 36			34		
L. leichmannii	34–36			34		
L. salivarius		34, 40				
L. casei	65, 92	92	17, 40, 45, 46, 48	65	65	
L. casei subsp. *pseudoplantarum*						85
L. curvatus			45, 46			85
L. sake						47, 54, 85
L. plantarum	13, 15, 18, 34, 36, 64	13, 15, 18, 34, 36, 40, 45, 64		18, 34, 82, 83	18, 82, 83	
L. brevis	18, 34, 36	18, 34, 36, 40		18	18	
L. buchnerii	34, 36	34, 36				
L. cellobiosus	34, 36, 78	78				
L confusus	28, 78	78		78	78	
L. fermentum	34–36, 78	78				
L. vermeforme	78	78			78	
L. viridescens	28, 34, 36, 78	78		78	78	
Leuconostoc						
L. cremoris	27, 28, 36, 50					
L. dextranicum	27, 28, 36, 38, 50					
L. lactis	27, 28, 36, 38, 49, 50					
L. mesenteroides	18, 19, 23, 27, 28, 36, 38, 50, 72, 78	19		18, 27, 78	18, 27, 78	
L. paramesenteroides	27, 28, 50			27	27	
L. oenos	18, 27, 28, 50					
Streptococcus						
S. agalactiae		63	106			
S. dysgalactiae			Garvie[b]			
S. uberis			32			
S. bovis		106	33, 105–107			
S. faecalis			10, 40, 100, 105, 106; Garvie[b]			
S. faecium			40; Garvie[b]	60		
S. lactis/S. cremoris	67		2, 10, 20, 30, 40, 52, 67, 105	2	2	
S. raffinolactis			30			
S. acidominimus			Garvie[b]			
S. millerii			Garvie[b]			
S. mutans		107, 108	6, 33, 40, 105, 107, 108			
S. mitior			105; Garvie[b]			
S. salivarius			6, 105; Garvie[b]			
S. sanguis			6, 105; Garvie[b]			
S. thermophilus		31	106			
Pediococcus						
P. acidilactici	3	3				
P. damnosus	3	3				
P. dextrinicus			3			
P. halophilus		3				
P. inopinatus	3	3				
P. pentosaceus	3, 18,[c] 39[c]	3, 18,[c] 39[c]		55[d]		
Bifidobacterium bifidum		40	16			
Aerobacter						
A. aerogenes	72, 74			68, 71	71	
A. cloacae				71	71	

TABLE 1—*Continued*

Taxon	Reference(s)					
	nLDH			iLDH		
	Forming D(−)-lactate	Forming L(+)-lactate	FDP-activated, forming L(+)-lactate	Forming D(−)-lactate	Forming L(+)-lactate	Lactate racemase
Escherichia						
E. coli	90, 91			22, 44, 58, 59, 71, 88, 89	44, 58, 71, 88	
E. freundii				71	71	
Hafnia sp.				71	71	
Klebsiella sp.				71	71	
Salmonella typhimurium				71	71	
Proteus vulgaris				71	71	
Serratia sp.				71	71	
Acetobacter peroxydans				12	12	
Pseudomonas						
P. aeruginosa				57	57	
P. natriegens				95	95	
Propionibacterium pentosaceum				66	66	
Staphylococcus						
Various species	42	42				
S. aureus	42	24, 42, 86, 87		87	80, 87	
S. epidermidis			41–43			
S. ureae						54
Acholeplasma laidlawii			69, 70			
Mycoplasma						
Various species	69	69				
M. gallisepticum					81[a]	
Bacillus						
B. caldolyticus		97[a]				
B. subtilis		110, 111				
Clostridium butylicum						9, 14
Butyribacterium rettgeri[e]	98, 102			101, 103, 104	101, 103, 104	
Butyrivibrio fibrosolvens			94[a]			
Selenomonas ruminantium	96	75				
Actinomyces viscosus			5			
Rothia dentocariosa			21			

[a] Specificity of lactate uncertain.
[b] Garvie, unpublished data.
[c] This taxon is called *Pediococcus cerevisiae* in this publication.
[d] This taxon is called *Leuconostoc mesenteroides* in this pulication.
[e] Now called *Eubacterium limnosum*.

D(−)- or L(+)-lactate are thought to have only a single nLDH, although on electrophoresis more than one area of enzyme activity has been detected in strains of different species (27, 30, 31, 32, 33). A maximum of four well-separated areas of LDH activity have been found in extracts of *Streptococcus faecalis* cells (Garvie, unpublished data). Only in *Streptococcus thermophilus* (31) is there any suggestion that the bands are different nLDH's. In other strains multiple banding is probably due to different forms of the same enzyme. It was found with both *S. faecalis* and *Streptococcus uberis* that changing the buffer in the developing solution from tris(hydroxymethyl)aminomethane (Tris)-chloride (pH 7.0) to acetate (pH 5.5) or 0.2 M phosphate (pH 7.0) affected all enzyme bands equally, nor were any differences detected when several concentrations of fructose 1,6-diphosphate (FDP) were used in these different buffers (Garvie, unpublished data). The majority of bacteria forming DL-lactate have two different nLDH's, which can be separated by gel electrophoresis of crude cell extracts and detected when the gels are developed with D(−)- or L(+)-lactate. In strains in which one or both nLDH's are active only with pyruvate, separate detection is more difficult but can usually be achieved by making use of some other property (see below).

D(−)-nLDH's. Most D(−)-nLDH's are reversible (13, 27, 34, 39, 49), but a few which use only pyruvate as substrate are known. In an early study of *Lactobacillus casei*, a D(−)-nLDH was partially purified. It reacted with lactate only when the substrate concentration was high (65). The D(−)-nLDH of *L. casei* is a minor enzyme of low activity, and the presence of a similar enzyme in *Lactobacillus salivarius* is possible because this species also forms traces of D(−)-lactate (85). A more significant irreversible

D(−)-nLDH is found in *E. coli*, and it has been examined in some detail (90, 91). *Aerobacter aerogenes* (72) and *Butyribacterium rettgeri* (98, 102) have D(−)-nLDH's which function with lactate only when NAD analogs are used as coenzymes.

L(+)-nLDH's. L(+)-nLDH's are found along with D(−)-nLDH's in most bacteria which form DL-lactate (see Table 1 for references), but some species form DL-lactate by using racemases (see below). L(+)-nLDH's are of two kinds, those which are activated by FDP and those which are not. The DL-lactate-forming lactic acid bacteria have L(+)-nLDH's which do not require FDP, and in many species these enzymes catalyze reversible reactions (13, 34, 39). Some lactobacilli have L(+)-nLDH's which react with lactate weakly or not at all, so that they can be detected after electrophoresis only with pyruvate. The L(+)-nLDH's of *Lactobacillus fermentum* and *Lactobacillus cellobiosus* were not reported when gels were developed with lactate (34) but were found with pyruvate (78); *Lactobacillus viridescens* and *Lactobacillus confusus* have nLDH's with similar properties (78).

Fructose 1,6-diphosphate-activated nLDH's. The other type of bacterial L(+)-nLDH, that activated by FDP, is found in most streptococcal species, in a few lactobacilli (notably *L. casei*), and in a few other bacteria (Table 1). Many of these nLDH's show an absolute requirement for FDP at physiological pH's (46, 106), although that of *Actinomyces viscosus* only requires FDP at low pyruvate concentrations (5). *Butyrivibrio fibrisolvens* is reported to have an nLDH activated by FDP (94). The specificity of the *Butyrivibrio* enzyme is not clear, but the reaction is faster with D(−)-lactate than with L(+)-lactate. If this is a D(−)-nLDH, it would be interesting, for no other FDP-controlled D(−)-nLDH is known. However, the enzymes of *B. fibrisolvens* require further study, and the isomer of lactate formed by cell extracts from pyruvate should be determined.

The FDP-activated L(+)-nLDH's are virtually nonreversible. Many streptococcal LDHs react weakly with lactate and NAD (10, 53, 106); NAD analogs have not been tried, but NAD analogs enable the nLDH of *Acholeplasma laidlawii* to react with lactate (88). It is not clear whether the FDP-activated nLDH of *Staphylococcus epidermidis* is reversible. Although it is called irreversible because it is active only with pyruvate as substrate in assays (43), it can be developed with lactate after gel electrophoresis (42).

Nicotinamide Adenine Dinucleotide-Independent LDHs (iLDH's)

The iLDH's are probably all flavin-containing proteins (see below) and are widely distributed in bacteria. They are more important to the survival of catalase-positive organisms, where they enable the bacteria to use lactate as a source of carbon (12, 57, 66, 75, 95), than in the lactic acid bacteria, where the function of the enzymes is still a matter of speculation (82). In these lactic acid bacteria the specificity of the iLDH's bears no relation to the type of lactate formed by the nLDH's. Several strains of *Leuconostoc* [D(−)-lactate-forming bacteria] have both L(+)- and D(−)-iLDH's, but the D(−)-iLDH is more active in vitro (27). The group N streptococci [L(+)-lactate-forming bacteria] can also have both D(−)- and L(+)-iLDH's (2). The iLDH's in the lactic acid bacteria are usually weak and are observed only when the methods used are designed for their detection (27), but some iLDH's in the lactobacilli were detected after electrophoresis of crude cell extracts when the gels were developed for nLDH's (34).

Some iLDH's are linked with electron transport and transfer. These enzymes are particulate, and some are known to be associated with cell membranes (22, 80). In *E. coli* and other *Enterobacteriaceae* the iLDH used in electron transport reacts with D(−)-lactate (59), but in *S. aureus* the electron donor is L(+)-lactate (80). iLDH's are found in bacteria which grow well aerobically (e.g., enterobacteria and staphylococci); however, they are also found in bacteria which are anaerobes (e.g., *Propionibacterium pentosaceum* [66]) and possibly in the strict anaerobe *Selenomonas ruminantium* (75).

Lactate Racemases

Although outside the scope of this review, the few bacteria in which lactate racemases have been found are listed in Table 1. Racemases in *Staphylococcus ureae* and *Lactobacillus sake* were observed in 1937 (54), and racemases have been shown in only a few additional species since that time. The racemases of *L. sake* (47), *Lactobacillus curvatus*, *L. casei* subsp. *pseudoplantarum* (85), and *Clostridium butylicum* (9, 14) have been isolated. In the lactobacilli the nLDH's form L(+)-lactate; the specificity of the LDH in *C. butylicum* is not known.

Difficulty in demonstrating two different LDHs in some species led several investigators to suggest that racemases were not infrequent in DL-lactate-forming bacteria. Early work with *L. casei* (65) detected a D(−)-nLDH but no L(+)-nLDH, although more than 90% of the lactate formed was L(+) when the culture was grown in glucose-containing media. It is now known that the assay conditions used in this early work prevented the action of the L(+)-nLDH present in the species because no FDP was used and the pH was too high. A lactate racemase was indicated in *S. aureus* (87), but more recent work

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has demonstrated a weak D(−)-nLDH in addition to the active L(+)-nLDH (42).

TYPE OF LACTATE FORMED BY BACTERIA

The isomer or isomers of lactate formed are used in the classification of the lactic acid bacteria (7). Strains are grown on a glucose-based medium, and under these conditions, the isomer or isomers of lactate formed indicate the nLDH's present in a species (85). Enzymic methods of determining lactate, which were not available to early workers, have shown small amounts of an isomer which was missed when lactate had to be extracted from culture supernatants. *L. casei*, long considered to be an L(+)-lactate former, produces small amounts of D(−)-lactate; thus, no *Lactobacillus* forms only L(+)-lactate. Streptococci, on the other hand, make only L(+)-lactate and have no D(−)-nLDH. One strain of *Streptococcus cremoris* has been reported to form DL-lactate and to contain both D(−)- and L(+)-nLDH's (67). Unfortunately, although the culture in this study was checked for various properties, single colonies were not purified and tested. It is possible that the enzyme preparations were made from a mixed culture. The D(−)-nLDH which was partially purified had an optimum pH of 8.5 for the reaction with pyruvate and NADH, which is unusually high; however, this might partially explain the value of 36:1 for the ratio of L-lactate to D-lactate formed. Activation of the L(+)-nLDH by FDP might also explain the high proportion of L(+)-lactic acid formed by the growing culture. If this strain is still available and proves to be a genuine DL-lactate former, it could be interesting for further studies on the influence of substrates and metabolites on the end products formed by *S. cremoris*.

All leuconostocs form D(−)-lactate from glucose (27). They have a single D(−)-nLDH and no L(+)-nLDH (27, 50). This latter enzyme has been reported from a strain of *Leuconostoc mesenteroides* (strain 39 of the University of Mainz) and from *Leuconostoc oenos* (18), but these L(+)-LDH's are not mentioned in a subsequent publication (38). The same strain 39 has been examined independently, and no L(+)-nLDH was found (Garvie, unpublished data). (A different strain 39, which is also designated NCDO 768 and ATCC 12291, has been used extensively in other work.) Strains of both *L. mesenteroides* and *L. oenos* were examined after growth in medium containing glucose and in medium containing glucose plus malate (19). D(−)-Lactate was formed from glucose, but DL-lactate was formed from glucose plus malate. The enzyme involved in malate utilization has been studied in several laboratories (1, 76) and forms L(+)-lactate (1). Pyruvate is not an intermediate in the reaction (76). No LDH is involved.

Other lactic acid bacteria form DL-lactate, but the ratio of the isomers can change at different times in cultures growing without pH control (26, 39, 85). *Pediococcus pentosaceus* (called *Pediococcus cerevisae* [26, 39]) and homofermentative lactobacilli (except *Lactobacillus plantarum*) have a high proportion of L(+)-lactate early in the growth cycle and form D(−)-lactate when the medium has a low pH.

When *P. pentosaceus* was grown in glucose-containing media, L(+)-lactate was produced preferentially while carbohydrate was plentiful (39). When the pH fell to about 5.0, the L(+)-lactate production stopped, and D(−)-nLDH became active. It was thought that the intracellular pyruvate concentration was below 0.5 mM. The pH optimum of the D(−)-nLDH changed with pyruvate concentration, from pH 8.0 at 5.0 mM to pH 3.6 at 0.5 mM. The optimum pH of the L(+)-nLDH remained at 4.5 to 6.0 irrespective of pyruvate concentration. The K_m at pH 5.4 calculated for the D(−)-nLDH was 0.15 mM, and the K_m was lower for the L(+)-LDH (1.0 mM). The influence of the environmental pH on the internal pH of the cells is not known. The low pH and low concentration of pyruvate prevailing as the culture entered stationary phase was thought to stimulate the production of D(−)-lactate at the expense of L(+)-lactate. There is not enough known about other species to determine whether changes in the ratio of L-lactate to D-lactate could be explained by a similar mechanism.

Certainly in *L. plantarum* the ratio of L-lactate to D-lactate formed when cultures are grown without pH control does not change much (26, 85). This species was observed to have different ratios of L(+)-nLDH to D(−)-nLDH in sonicated cells in different experiments (13); the conditions of growth causing these differences were sought but not found.

Lactobacillus ruminis (79) has two active nLDH's in freshly broken cells, as judged by assay and gel electrophoresis. Cell extracts in 0.2 M Tris-maleate buffer formed 40% D(−)-lactate at pH 8.3 but only 10 to 18% at pH values below 7.0. As growing cultures form over 90% L(+)-lactate, it seems that at acid pH's the D(−)-nLDH has little activity.

In other species one LDH may be in excess of the other. This has been proved in *L. casei* (85) and appears to be the case in *L. viridescens*, in which the D(−)-nLDH is predominant and only small amounts of L(+)-lactate are formed by a growing culture (26, 78). *L. viridescens* nLDH's have been examined only under limited conditions in crude cell extracts, so that observations

on the relative activities of the two LDHs must be treated with caution. As lactate racemases are induced by L(+)-lactate (85), the few lactobacilli in which racemases occur form L(+)-lactate first.

Lactate production in other genera has not been studied as well as in the lactic acid bacteria. A recent survey of the staphylococci (42) shows that L(+)-, D(−)-, and DL-lactate are formed by different species. Although some of the properties of the nLDH's have been determined, nothing is known about the relative amounts of D- and L-lactate in the supernatants of cultures of DL-lactate formers, some of which form only small amounts of lactate.

PURIFICATION AND PROPERTIES OF BACTERIAL LDHs

nLDH's

Growth of cells. The first consideration is to obtain the maximum cell yield with a maximum content of enzyme. With the lactic acid bacteria this can normally be achieved by using an adequate glucose-based complex nutrient medium at a suitable temperature. The growth conditions selected vary; e.g., *L. casei* has been grown at 30°C (48), 34°C (46), 37°C (17), and 45°C (65). Batch cultures can be used (49, 65, 100), but higher yields of cells are obtained when a constant pH is used (10, 46, 48, 52); the nLDH does not appear to be affected. Some workers have preferred carbohydrates other than glucose. *L. oenos* was grown on ribose (50), *S. cremoris* was grown on lactose (52), *Streptococcus mutans* was grown on mannitol (6), and *A. viscosus* was grown on sucrose (5). Lactic acid bacteria should not be grown aerated; a closed vessel is satisfactory, but some strains have been grown with reduced oxygen (16, 46, 48, 100).

Other bacteria have been grown under a variety of conditions, depending on the species, and growth conditions may have more influence on the nLDH's of these bacteria than they do on the nLDH's of the lactic acid bacteria. The nLDH of *Bacillus caldolyticus* has different properties when grown aerated with or without brain heart infusion (97). In *Bacillus subtilis* formation of the nLDH was stopped if the strain was grown aerated in a glucose minimal salts medium (111), but *Rothia dentocariosa* can be grown aerated (21). nLDH's have been purified from both *E. coli* (90) and *A. aerogenes* (74). The former was grown on maltose, but the latter was grown on glucose, both statically and anaerobically.

Cells are normally harvested at the end of the logarithmic phase of growth, and the nLDH is present in quantity, providing that the conditions of growth selected have ensured high lactate production. The specific activities of nLDH's per milligram of cell protein in broken cells of lactic acid bacteria vary widely. In one laboratory, the figures ranged from 0.3 to 40.0 when 24 strains of several species were studied (40); similar variation was observed in other work (Garvie, unpublished data). The wide variation may be explained in part by the fact that these workers used standard assay conditions rather than optimal conditions for each enzyme in the study.

Breaking cells. Wet packed cells can be stored deep frozen (5, 30, 41, 46, 49, 72, 108). This technique is often used as a matter of convenience, but in this laboratory deep freezing of cells before breakage is routine because thawed cells break more readily than fresh cells. Cells can also be freeze dried (100) or acetone dried (111).

The buffer used for suspending the cells before breaking can be critical to the stability of the LDH. Phosphate buffers are used most often, but the concentrations range from 2 mM (13) to 100 mM (34, 106). Some streptococcal nLDH's are stable at 100 mM (pH 7.0) but lose activity rapidly and irreversibly at 10 mM (pH 7.0) (33). Tris-chloride has been used at different concentrations (23, 87), and 0.1 M acetate (pH 5.5) was selected for *Lactobacillus* sp. cells (46). The pH of the buffer is also important, and pH 7.0 has been used widely. *S. aureus* was broken in 0.05 M Tris at pH 8.2 (87) and also in 0.05 M phosphate at pH 6.5 (24).

Reducing agents have been used to stabilize the nLDH's in some instances (23, 41, 50, 106), but this appears to be a precaution rather than a necessity, as other laboratories which have used the same species have not used reducing agents. Reducing agents were used with *E. coli* (23) and are required to maintain the enzyme in a fully active form. Ethylenediaminetetraacetate was included when cells of *L. casei* were broken (40), but it has not been used in other laboratories (46). Sodium chloride has been included in buffers when cells of lactic acid bacteria and *A. laidlawii* were broken (27, 70), but it may not be necessary.

All available methods of mechanical breakage have been used, and none is reported as being destructive to any nLDH. Normally, mechanical breakage has been used, although *B. subtilis* cells were digested with lysozyme (111). Broken cell preparations are clarified by high-speed centrifugation, and since the nLDH's are not sedimented, it is believed that they are all cytoplasmic enzymes.

Assay of nLDH's. Assay conditions which are selected in advance of an understanding of an enzyme may be far from ideal. Table 2 shows the conditions which have been selected to assay different nLDH's. nLDH's react with pyruvate and NADH in a suitable buffer at room temperature; FDP is the only additional compound known to be essential, and it is essential only for certain L(+)-nLDH's. A number of nLDH's have been assayed by using lactate and NAD, but oxidation of lactate requires an alkaline pH and a high concentration of substrate. The FDP-activated nLDH's in *Lactobacillus* species (46) do not oxidize lactate. In contrast, the nLDH's of *S. epidermidis* (43) and the streptococci do oxidize lactate, but the reaction is weak. All streptococcal LDHs known actively reduce pyruvate at pH 5.5. As the ratio of activity at pH 5.5 to activity at pH 7.0 increases, the ability to oxidize lactate decreases (Garvie, unpublished data), and nLDH's inactive at pH 7.0 are virtually unidirectional, reacting only with pyruvate at acid pH's (46). It has also been observed that the nLDH's of *S. uberis* strains which are stimulated by rather than dependent on FDP oxidize lactate faster than the nLDH's of other strains (32).

The inhibitory effects of a number of common buffer compounds on the FDP-activated nLDH's have been reported (see below). Earlier workers (6, 100) chose phosphate buffers without knowing that PO_4^{3-} was not an inert ion in the system. Phosphate is stimulatory to the nLDH of *S. faecalis* under some conditions (see below) but inhibitory to the nLDH of *S. mutans* (Garvie, unpublished data). The high level of FDP used in the work with *S. mutans* (6) was probably required to overcome the inhibitory effect of phosphate.

NADH oxidases (EC 1.6.99.3) may interfere in assays of nLDH's when pyruvate is used as substrate. nLDH is the more active enzyme in most strains and is detected at a dilution at which NADH oxidase (if present) is inactive. In one strain of *L. mesenteroides*, NADH oxidase prevented the assay of active D(−)-nLDH in crude cell extracts (27). In *L. viridescens* L(+)-nLDH, which does not react with lactate, was difficult to locate after gel electrophoresis because it was close to an active NADH oxidase (78). A weak NADH oxidase is detected if for some reason a high level of crude cell extract is used to assay an nLDH, as was the case with *S. thermophilus* (31) and *Streptococcus agalactiae* (63). The strain of *S. agalactiae* in which the oxidase was demonstrated has an active FDP-activated nLDH (Garvie, unpublished data), which was apparently inactivated when the strain was first examined.

Electrophoresis. Reversible nLDH's can be readily detected by using lactate and NAD with phenazinemethosulfate and Nitro Blue Tetrazolium to color the reaction (27, 34, 43, 49). These enzymes can be separated and then stained by using buffers with pH's above 8.0; Tris-maleate or Tris-chloride is satisfactory. nLDH's can also be separated at pH 7.0, and this pH has been used for FDP-activated nLDH's, with Tris-phosphate as gel and tank buffer (30, 32, 33); glycyl-glycine buffer (pH 7.5) was used for the LDHs of *L. casei* (46). The enzymes can be detected in any suitable buffer at low pH; FDP-activated enzymes and other enzymes which react weakly with lactate can be treated with pyruvate and NADH and then located with phenazinemethosulfate and Nitro Blue Tetrazolium (27, 30, 90). Developing with lactate has the advantage of locating D- and L-LDHs separately; usually there is some difference between D- and L-nLDH's in DL-lactate-forming species which enables both enzymes to be located even when lactate cannot be used. In some species of lactobacilli, L(+)-nLDH can be detected only when pyruvate is used as substrate, but D(−)-nLDH can be detected with either pyruvate or lactate (78). In *L. casei* neither the D(−)- nor the L(+)-nLDH can be detected with lactate, but only the L(+)-nLDH requires FDP and only the D(−)-nLDH is active at pH 7.0 (Garvie, unpublished data). The experimental conditions were the same as those used for various streptococci (30). There is evidence that the D(−)-nLDH in *L. casei* is not unidirectional (65), and the failure to detect it with lactate after gel electrophoresis was probably due to the low concentration of this nLDH in cell extracts (85) and also to the high concentration of lactate required.

Purification of nLDH's. nLDH's of all types have been purified to the extent that electrophoresis shows a single band of protein. Two LDHs have been crystallized, the D(−)-nLDH of *L. mesenteroides* (23) and the L(+)-nLDH of *B. subtilis* (111). None of the FDP-activated nLDH's has been crystallized, but as they depend on FDP or PO_4^{3-} to maintain activity in solution, it is possible that they would be irreversibly inactivated if they were crystallized.

Most purification procedures have followed standard techniques, and phosphate buffer at a pH somewhere between 6.0 and 7.5 and a concentration somewhere between 0.01 and 1.0 M is a common choice of solvent. *L. mesenteroides* nLDH was purified by using Tris buffer at pH 7.5 (23), and *Lactobacillus* LDHs were purified in 0.01 M acetate buffer at pH 5.5 with differing

TABLE 2. *Assay conditions used with nLDH's.*

Taxon	Reference	Buffer			Concn (mM) of:			Buffer			Concn (mM) of:			Comments
		Composition	pH	Concn (mM)	NADH	Pyruvate	FDP	Composition	pH	Concn (mM)	NAD	Lactate	FDP	
Lactobacillus acidophilus	45							Glycine	9.0	0.4	3.9	200.0		1.7% hydrazine added
L. casei	17	Histidine	Range	0.08	0.3	4.0	1.2							Mn^{2+} required
L. casei	46	Acetate	4–5.8	0.08	0.65	17.0	3.0							10 mM $MnSO_4$ added
L. casei	39	Acetate	5.4	0.05	0.167	10.0	0.1							
L. casei	40	Tris-maleate	5.4	0.025	0.1	10.0	1.0							
L. casei		Acetate	5.4	0.025	0.1	10.0	Varied							
L. casei	48	Acetate	5.4	0.05	0.167	10.0	0.1							
L. curvatus	46	Imidazole	6–7.5	0.04	0.65	17.0	3.0							10 mM $MnSO_4$ added
L. plantarum	13							Tris	8.5	0.01	0.75[a]	20.0[D(−)]		
									7.5	0.01	0.75[a]	20.0[L(+)]		Ca^{2+} lactates
L. plantarum	45							Glycine	9.0	0.4	3.9	200.0		1.7% hydrazine added
L. plantarum	64	Phosphate	7.0	0.167	0.07	3.3								
Various species	34							Tris-chloride	8.0	0.2	2.0	100.0		
	35	Tris-chloride	8.0	0.2	0.2	20.0		Tris-chloride	8.0	0.2	2.0	100.0		
Leuconostoc														
L. mesenteroides	23	Phosphate	7.5	0.1	0.02–0.1	0.13–0.67		Tris-chloride	8.7	0.1	3.0–1.0	33.0–6.7		
L. lactis	49	Tris-chloride	7.6	0.016	0.2	20.0		Tris-chloride	8.0	0.016	2.0	100.0		
Various species	27	Phosphate	7.0	0.03	0.15	0.33		Tris-chloride	8.3	0.1	0.75	100.0[b] 25.0[c]		
	40	Tris-maleate	7.5	0.016	0.2	0.5		Tris-maleate	8.0	0.016	1.6	10.0		30°C
	38	Tris-maleate	7.5	0.017	0.2	0.5		Tris-maleate	8.0	0.017	1.6	10.0		
			5.4	0.017	0.2	0.5								
Streptococcus														
S. faecalis	100	Phosphate	6.2	0.1	0.1	5.0	1.0							
S. cremoris	52	Triethanolamine	8.0	0.083	0.13	6.6								
S. cremoris	20	Triethanolamine	7.0	0.033	0.16	6.7	0.033							
S. lactis	10	Tris-maleate	6.9	0.09	0.167	10.0	1.0							
S. mutans	6	Phosphate	6.2	0.1	0.1	10.0	20.0							
S. mutans	108	Phosphate	6.2	0.067	0.08	10.0								
S. mutans/S. bovis	107	Tris-chloride	7.0	0.067	0.11	33.0	0.33							
Various species	106	Tris	7.2	0.017	0.08	27.0	1.0	Tris	7.4	0.017	0.33	333.3	1.0	Other compounds added
	30	Tris-chloride	7.0	0.067	0.15	3.3	0.5	Tris-chloride	7.5	0.067	0.75	100.0	0.5	
		Acetate	5.5	0.067	0.15	3.3	0.5							
Pediococcus pentosaceus	39	Tris-maleate[d]						Tris-maleate	8.2	0.025	2.0	10.0		30°C
Bifidobacterium bifidum	16	Tris-chloride	7.4	0.18	0.3	7.3	0.73							
Aerobacter aerogenes	74	Phosphate	5.7	0.1	0.15	5.0								
	72	Phosphate	7.0	0.11	0.12	6.7								
		Tris-chloride	7.1	0.033										
Escherichia coli	90 91	Phosphate	7.5	0.1	0.33	30.0		Pyrophosphate	8.9	0.03	0.7	24.0		

Pediococcus pentosaceus	39	Acetate	5.5	0.067	0.15	3.3	0.5	Tris-maleate	8.2	0.025	2.0	10.0		30°C
		Tris-maleate[d]												
Bifidobacterium bifidum	16	Tris-chloride	7.4	0.18	0.3	7.3	0.73							
Aerobacter aerogenes	74	Phosphate	5.7	0.1	0.15	5.0								
	72	Phosphate	7.0	0.11	0.12	6.7								
		Tris-chloride	7.1	0.033										
Escherichia coli	90	Phosphate	7.5	0.1	0.33	30.0								
	91							Pyrophos- phate	8.9	0.03	0.7	24.0		

TABLE 2. *Continued*

Taxon	Reference	Buffer			Concn (mM) of:			Buffer			Concn (mM) of:			Comments
		Composition	pH	Concn (mM)	NADH	Pyruvate	FDP	Composition	pH	Concn (mM)	NAD	Lactate	FDP	
Staphylococcus														
S. aureus	24	Phosphate	6.5	0.05	0.136	2.5								
	86							Tris-chloride	7.5	0.032	0.1	7.5		4.2 mM KCN added
S. epidermidis	43	Imidazole	6.3	0.082	0.57	12.3	0.8							
	41	Phosphate	5.6	0.1	0.58	2.3	0.4							
Acholeplasma laidlawii	70	Tris-chloride	7.2	0.1	0.15	0.5	1.0	Tris-chloride	8.0	0.1	2.0[e]	10.0	1.0	
Bacillus														
B. caldolyticus	97	Phosphate	6.2	0.1	1.0	9.0								
B. subtilis	110	Tris	8.0	0.05	0.16	6.0								
Butyribacterium rettgeri	98	Phosphate	6.2	0.1	0.1	Variable		Tris-chloride	8.0	0.1	5.0[f]	100.0		
Butyrivibrio fibrisolvens	94	Tris-chloride	7.0	0.067	0.17	33.3								
Selenomonas ruminantium	96	Phosphate	7.0	0.02	0.25	16.6								
	75	Tris-chloride	7.0	0.05	0.3	10.0		Tris-chloride	8.5	0.05	0.3	10.0		
Actinomyces viscosus	5	Phosphate	6.2	0.1	0.1	5.0								
Rothia dentocariosa	21							Tris	7.2	0.025	1.0	100.0	1.0	5.0 mM dithiothreitol and 5.8 mM $MgSO_4$ added
Mammal	73	Phosphate	7.0	0.1	0.117	0.33								

[a] 3-Acetylpyridine NAD.
[b] Sodium DL-lactate.
[c] Calcium D(−)-lactate.
[d] Varying conditions for D(−)- and L(+)-nLDH's.
[e] NAD analog.
[f] Acetylpyridine NAD.

concentrations of NaCl (45). Nucleic acids have been precipitated with protamine or streptomycin sulfate, although extracts of *S. faecalis* were treated with deoxyribonuclease (100) and extracts of *B. subtilis* were treated with deoxyribonuclease and ribonuclease (111). Proteins have been precipitated selectively with $(NH_4)_2SO_4$, but heat has been used with some *Lactobacillus* extracts (45), streptococci (100), and *Acholeplasma* (70), with the amount of heating depending on the strain. Further purification has been achieved with column chromatography, and a variety of conditions have been satisfactory (5, 6, 10, 13, 23, 35, 38-40, 45, 46, 48, 49, 52, 64, 67, 70, 72, 87, 90, 97, 100, 104, 111). Diethylaminoethyl-cellulose, diethylaminoethyl-Sephadex, and Sephadex G200 have been used most often, but other materials have also been tried; these other materials have included Sepharose 6B (39), hydroxyapatite (39, 49), calcium phosphate gel (64, 70, 100, 102, 111), diethylaminoethyl protein (10, 48), and Ecteola cellulose (111). Agarose permeation chromatography (5) and preparative electrophoresis (40, 49) have also been used successfully.

Recently, affinity chromatography has been introduced as a final step in purification. Various gels have been successful. *L. casei* and *L. curvatus* nLDH's were purified on Sepharose 4B-oxamate (46); the same gel was used for *S. epidermidis* nLDH (41). *L. plantarum* and *Lactobacillus acidophilus* L(+)-nLDH's were purified on oxamate-Sepharose 6B (45); adenosine 5'-monophosphate-Sepharose 4B (40) and oxamate-agarose affinity resin (48) have been used for *L. casei* nLDH, and oxamate-Bio-Gel A has been used for *Streptococcus lactis* nLDH (10). All of these nLDH's form L(+)-lactate, but neither the D(−)-nLDH of *L. mesenteroides* nor the D(−)-nLDH of *L. plantarum* was retained by a Sepharose-linked oxamate gel (56). Such a gel was used in the preparation of the L(+)-nLDH's of some lactic acid bacteria and *B. subtilis*. The FDP-activated nLDH of *S. faecalis* bound to oxamate gels when NADH or NADH and FDP were present.

Many workers have examined only a single strain with a single nLDH, but a comparison of techniques for handling different strains is possible (35, 45). Some bacteria contain both L(+)- and D(−)-nLDH's, and methods to separate the enzymes need to be selected. This has been done in the case of *L. plantarum* (13, 64), where the enzymes were partially separated by $(NH_4)_2SO_4$ precipitation, the D(−)-nLDH was inactivated by heat, and the L(+)-nLDH was not eluted from diethylaminoethyl-cellulose with 0.8 M NaCl. Affinity chromatography (56) can also be used. The two nLDH's of *P. pentosaceus* were separated by eluting different columns with buffers of different pH values (39). *L. casei* has a weak D(−)-nLDH which was partially purified (65), but more recently workers have not looked for this enzyme as their work has concentrated on the more active L(+)-nLDH and the D(−)-nLDH has been lost somewhere in the procedure.

Specific activity after purification. The specific activities of purified preparations are shown in Table 3. Wide variations are found and do not appear to be explained by differences in assay conditions. One preparation of the L(+)-nLDH of *L. casei* had a specific activity of only 445, but the specific activities in two other preparations were more than 2,000. Some preparations have very low specific activities, notably those of *P. pentosaceus* (39). This species grows rapidly and lowers the pH of the medium to 3.8 to 3.9. It would be expected to contain nLDH's at least as active as those of other lactic acid bacteria.

pH optima. Information on the optimum pH values of the nLDH's is shown in Table 4. The interaction of pH and the effects of FDP and/or Mn^{2+} on the activities of the nLDH's of *L. casei* (40, 46, 48) and *L. curvatus* (46) in vitro is complex. The effect of Mn^{2+} on the streptococcal nLDH's has only been studied for *S. faecalis* (where Mn^{2+} activation was found) and for *S. lactis* (where Mn^{2+} had no effect) (10). Estimations of the optimum pH's of streptococcal nLDH's are probably less complicated than estimations for *Lactobacillus* nLDH's.

The optimum pH for lactate oxidation is usually higher than the optimum pH for the reaction with pyruvate. The nLDH in strains of *L. mesenteroides* was found to have an optimum pH of 9.8 for the reaction with lactate (18). This has been confirmed for strain 39 (Garvie, unpublished data), but the LDH of another strain (strain 99) has greater activity at pH 8.3 than at pH 9.8 (Garvie, unpublished data).

A. viscosus contains an L(+)-nLDH activated by FDP. This enzyme is distinguished from other similar LDHs by having an optimum pH of 8.5 for oxidation of lactate (5).

The range of pH values in which nLDH's show activity is wide, from pH 4.5 for FDP-activated L(+)-nLDH of *L. casei* with pyruvate (46) to pH 9.8 for D(−)-nLDH of *L. mesenteroides* with lactate (18). The optimum pH for the physiological reaction (pyruvate → lactate) is often below 7.0. Many of the enzymes studied are from lactic acid bacteria. It has been estimated that the internal pH of *L. plantarum* is 5.4 (39). If other lactic acid bacteria have similar pH's in their cells, it would be reasonable to expect enzymes to be active at acid pH values.

TABLE 3. *Specific activities of purified nLDH's*

Taxon	Reference	Sp act (U/mg)	Recovery (%)	LDH type
Lactobacillus				
L. acidophilus	45	2,350.0	48	L(+)
	35	38.3	16.3	D(−)
	35	11.6	19.8	L(+)
	35	12.2	14.9	L(+)
L. casei	65	69.3	21.0	D(−)
	46	2,320.0	6.0	L(+)
	40	2,291.7	4.8	L(+)
	48	445.0	9.6	L(+)
L. curvatus	46	2,030.0	40.0	L(+)
L. jensenii	35	123.5	3.0	D(−)
L. leichmannii	35	260.0	13.8	D(−)
L. plantarum	13	466.7		D(−)
	13	436.4		L(+)
	45	2,460.0	22	L(+)
	64	545	12	D(−)
	64	265	38	L(+)
L. fermentum	35	330.0	26.2	D(−)
Leuconostoc				
L. mesenteroides	23	1,510	18	D(−)
L. lactis	38	5.27	11	D(−)
	49	126.6	32	D(−)
Streptococcus				
S. faecalis	100	158.98	38	L(+)
S. cremoris	52	800.0	30	L(+)
	67	67.5	0.87	D(−)
	67	111.0	5.1	L(+)
S. lactis	10	1,400.0	33.0	L(+)
S. mutans	6	255.0	44.0	L(+)
	2	3.2	20.0	L(+)
Pediococcus pentosaceus	39	1.57	19.0	D(−)
	39	1.8116	28.0	L(+)
Aerobacter aerogenes	74	1,000.0		D(−)
	72	243		D(−)
Escherichia coli	90	250	4.6	D(−)
Acholeplasma laidlawii	70	777.0	36.0	L(+)
Bacillus				
B. caldolyticus	97	184.0	12.0	?
B. subtilis	110	11×10^3		L(+)
Actinomyces viscosus	5	212.0	58.8	L(+)
Mammal (muscle)	4	574	103	L(+)
Mammal (heart)	4	353	200	L(+)

Kinetics of nLDH's. Kinetic data for some nLDH's are shown in Table 5. These include values for four FDP-activated nLDH's which show activity in the absence of FDP under some conditions. When the enzymes are affected by FDP, kinetic measurements are complicated by having three variables acting on the activity of an enzyme. FDP has been found to affect the K_m values for both pyruvate and NADH for the nLDH's of *S. lactis* (10), *S. faecalis* (100), *S. mutans* (pyruvate only) (6), and *A. laidlawii* (70). The complicated kinetics of the nLDH's of *L. casei* and *L. curvatus* have been shown in detailed studies of these enzymes (46).

Unusual FDP saturation curves were found with the FDP-activated nLDH of *S. epidermidis* (41). The assay used 0.1 M phosphate buffer, and the effect of phosphate on enzyme activity is not known. Additional study is necessary before these curves can be explained. Nothing similar has been reported for any other LDH.

FDP-activated L(+)-nLDH's, as well as L(+)- and D(−)-nLDH's not affected by FDP, have complex kinetics. Sigmoidal plots for substrate are often found, and Hill plots give values near 2. These values are interpreted to mean that the enzymes have multiple binding sites and have been found for bacteria as diverse as *S. mutans* (6), *L. casei* (46), *A. viscosus* (5), *B. rettgeri* (102), *E. coli* (91), *A. aerogenes* (74), *S. ruminantium* (96), and *L. mesenteroides* with lactate as substrate (23). Hill plots also indicate multiple

TABLE 4. *Optimum pH values of nLDH's*

Taxon	Reference	Optimum pH		Comments
		Pyruvate	Lactate	
L(+)-LDHs				
Lactobacillus acidophilus	35	5.3	7.8	
Lactobacillus plantarum	13		7.5	
	64	6.0		
Pediococcus pentosaceus	39	4.5–6.0	7.6	
Staphylococcus aureus	87		8.2	
Bacillus caldolyticus	97	—[a]	—	
Bacillus subtilis	110	6.0	7.2	
FDP-activated L(+)-LDHs				
Lactobacillus casei	46	4.5		Raised by added FDP and Mn^{2+}
	48	5.5		Saturated with FDP
	40	5.5		Broadened by Mn^{2+}
Lactobacillus curvatus	46	4.5		Raised by added FDP and Mn^{2+}
Streptococcus cremoris	53, 67	8.0	8.0	Weak activity without FDP
	53, 67	5.0–8.0	7.5	With FDP
Streptococcus lactis	10	8.0		Weak activity without FDP
	10	6.9–7.0		With FDP
Streptococcus mutans	6	5.5–7.0		
Staphylococcus epidermidis	41	5.6		
Acholeplasma laidlawii	70	6.5		Without FDP
	70	7.0		With FDP
Actinomyces viscosus	5	5.0–6.2	8.5	Optimal activity in phosphate
D(−)-LDHs				
Lactobacillus acidophilus	35	7.3	7.8	
Lactobacillus jensenii	35	7.8	8.2	
Lactobacillus leichmanii	35	7.6	8.0	
Lactobacillus plantarum	13		8.5	
	64	6.0		
Lactobacillus fermentum	35	8.6	8.6	
Leuconostoc lactis	38	6.6–8.0	8.0	Changes with pyruvate concentration
	49	7.6	8.0	
Pediococcus pentosaceus	39	3.6 or 8.0	9.6	Changes with pyruvate concentration
Escherichia coli	91	6.4–7.5		
Mammal (muscle)	77	7.0	8.6	

[a] —, pH affected by growth medium.

binding sites for FDP on the nLDH of *S. lactis* (10). Coenzyme binding is less complex than substrate binding, and K_m values have been calculated from graphs of reciprocal plots (6, 10, 46). However, more than one binding site for NADH was indicated for the nLDH of *A. viscosus* (5).

Inhibitors and activators. A summary of the information found in the literature is given in Tables 6 and 7. The diverse conditions used by different laboratories make comparisons among the different nLDH's difficult. The effect of oxamate is relevant to the use of affinity chromatography in the purification of enzymes. Oxamate is an inhibitor of the D(−)-nLDH of *L. mesenteroides* (23), but this enzyme does not bind to an oxamate affinity column (56). The D(−)-nLDH of *L. plantarum* is unaffected by oxamate (13), and it also does not bind to the gel (23).

Phosphate is a general inhibitor of FDP-dependent L(+)-nLDH's (2, 10, 30, 32, 33, 70) and is apparently competitive with FDP in some cases. However, the reactions of the enzymes with phosphate and FDP are not clear. Some nLDH's which are sensitive to FDP are little affected by phosphate (33), and phosphate may even be stimulatory. This has been observed with *S. faecalis* LDH at neutral pH (Garvie, unpublished data). The inclusion of 20 mM phosphate in an assay containing 3.3 mM pyruvate and 0.5 mM FDP increased the activity by 10 to 30%. When the FDP concentration was decreased to 0.05 mM, phosphate stimulation was still detected. Phosphate stimulation was also observed with the nLDH's of some *Streptococcus bovis* and *S. uberis* strains (32, 33), and the LDH of *A. viscosus* (5) showed maximum activity in phosphate buffer. Phosphate has no effect on the nLDH's of *S. thermophilus* which

TABLE 5. *Information on the kinetics of some nLDH's*

Taxon	Reference	Assay conditions: Buffer composition	Buffer concn (M)	Buffer pH	Temp (°C)	K_m (mM): Pyruvate	Lactate	NADH	NAD	Comments
L(+)-LDHs										
Lactobacillus acidophilus	35	Tris-maleate	0.05	7.8	25		360.0			
				5.3		1.5		0.069		
		Tris-maleate	0.05	7.8	25		1,660.0			
				5.3		2.7		0.074		
Lactobacillus plantarum	13	Tris	0.01	7.5	25		55.0			
				8.0		0.37				
	64	Phosphate	0.2	5.4	25	0.5		0.02		
Pediococcus pentosaceus	39	Tris-maleate	0.025	7.4	30	8.0		0.04		
				5.4		1.0		0.02		
		Tris-maleate	0.025	8.2	30		67.0		5.0	
Bacillus caldolyticus	97	Phosphate	0.1	6.2	33	3.0		0.045		Figures given for other temperatures
Bacillus subtilis	110	Phosphate	0.05	7.2	25		30.0		0.9	
D(−)-LDHs[a]										
Lactobacillus acidophilus	35	Tris-maleate	0.05	7.8	25		1,250.0			
		Tris-maleate		7.3		4.6		0.16		
Lactobacillus jensenii		Tris-maleate	0.05	8.2	25		70.0			
		Tris-maleate		7.8		7.2		0.166		
Lactobacillus leichmannii		Tris-maleate	0.05	8.0	25		70.0			
		Tris-maleate		7.6		1.2		0.071		
Lactobacillus plantarum	13	Tris	0.01	8.5	25		19.0			
	64	Phosphate	0.2	8.0		4.7		0.027		
			0.2	5.4		0.59		0.027		
Lactobacillus casei	65	Phosphate		5.9		10.0		0.001		Pyruvate/lactate equilibrium constant, 4.5×10^{-13}
Lactobacillus fermentum	35	Tris-maleate	0.05	8.6	25		20.0			
			0.05	8.6		1.9		0.1		
Leuconostoc lactis	38	Tris-maleate	0.017	8.0			20.0		2.9	
			0.017	7.5		0.33		0.15		
			0.017	5.4		0.05		0.02		
	49	Tris-chloride	0.016	7.6		2.0		0.22		
			0.016	8.0			50.0		12.5	
Leuconostoc mesenteroides	23	Phosphate	0.1	7.5	25	1.0		0.067		
		Tris-chloride	0.1	8.7	25		Curved		2.2	
Pediococcus pentosaceus	39	Tris-maleate	0.025	7.4	30	0.67		0.01		
			0.025	5.4		0.15		0.0083		
			0.025	8.2			30.0		1.05	
Selenomonas ruminantium[b]	96	Phosphate	0.025	7.0						
FDP-activated L(+)-LDHs[c]										
Lactobacillus casei	46	Acetate	0.08	4.8				0.045		
Lactobacillus curvatus		Acetate	0.08	4.8				0.033		
Streptococcus lactis	10	Tris-maleate	0.5	6.9		15		0.08		0.5 mM FDP present
		Triethanolamine	1.0	7.9			100		2.4	1.0 mM FDP present
				8.0			500		8.0	No FDP
Streptococcus cremoris	53	Triethanolamine	0.1	8.0		77		0.4		No FDP
Streptococcus mutans	6	Phosphate	0.1	6.2				0.032		Unaffected by FDP
Mammal (H_4)	4	Tris	0.2[d]	7.0	28.5	0.14		0.024		
Mammal (M_4)	73	Phosphate	0.1	7.5	25	1.0		0.0107		
Mammal (H_4)	4	Tris	0.2[d]	7.0	28.5		17.0		0.1	
Mammal (M_4)	73	Phosphate	0.1	7.5	25		25.0		0.253	

[a] The kinetics of the D(−)-LDHs of *Escherichia coli*, *Aerobacter aerogenes*, and *Butyribacterium rettgeri* are anomalous (74, 91, 98).

[b] The K_m reciprocal plots are nonlinear.

[c] The kinetics of these enzymes are complex, and curved reciprocal plots are often obtained. Various studies have been made (5, 6, 10, 40–42, 46, 48, 70, 100). There may be multiple binding sites for pyruvate but only a single one for NADH.

[d] Ionic strength.

are not FDP activated (31).

The stimulatory effect of FDP is unique. The effects of other phosphorylated compounds formed during glycolysis have been tested on the LDHs of several species, including *Streptococcus* (various species) (106), *S. faecalis* (100),

TABLE 6. *Inhibitor and stimulatory effects of some compounds on nLDH's*[a]

Substance	Taxon	Reference	Type of LDH	Effect on enzyme
Oxamate	*Lactobacillus casei*	46	L(+) (FDP)	Inhibitory; affects substrate binding
	Lactobacillus plantarum	13	L(+)	50% inhibition at 0.5 mM for lactate oxidation and at 0.15 mM for pyruvate reduction
			D(−)	No inhibition at 8.0 mM for either reaction
		64	D(−) and L(+)	Inhibition at 4 mM
	Lactobacillus (various species)	35	D(−) and L(+)	No inhibition detected at 5.0 mM
	Leuconostoc lactis	38	D(−)	50% inhibition at 1.5 mM (pH 5.4) for pyruvate reduction
				No inhibition at 8.0 mM for lactate oxidation
	Leuconostoc mesenteroides	23	D(−)	50% inhibition at 3.0 mM (pH 7.5) for pyruvate reduction
	Streptococcus mutans	6	L(+) (FDP)	Inhibitory; did not substitute for pyruvate
		108		60% inhibition at 3.3 mM
	Pediococcus pentosaceus	39	D(−)	50% inhibition at 5 mM (pH 5.4) for pyruvate reduction
			L(+)	50% inhibition at 0.5 mM (pH 5.4) for pyruvate reduction and 50% inhibition at 7.0 mM (pH 7.0) for pyruvate reduction
			D(−), L(+)	No inhibition at 8.0 mM for lactate oxidation
	Escherichia coli	91	D(−)	Inhibition at 0.3 mM (pH 7.5) in phosphate buffer (mixed type of inhibition)
	Butyribacterium rettgeri	102		No inhibition at 3 mM
Oxalate	*Leuconostoc lactis*	38	D(−)	50% inhibition at 1.1 mM (pH 5.4)
	Pediococcus pentosaceus	39	L(+)	50% inhibition at 0.5 mM (pH 5.4) for pyruvate reduction
α-Keto butyrate	*Streptococcus mutans*	6	L(+) (FDP)	Changes in Hill values for pyruvate from 1.7 to 1.1
	Escherichia coli	91	D(−)	Poor substitute for pyruvate; alters pyruvate kinetics
	Acholeplasma laidlawii	70	L(+) (FDP)	Effective substrate using pyruvate binding sites but with lower affinity
	Bacillus subtilis	110	L(+)	Substrate of low affinity in 0.05 M phosphate, pH 6.0
	Butyribacterium rettgeri	102		Substrate when pyruvate concentration is low, otherwise inhibitory; alters pyruvate kinetics
Oxaloacetate	*Bacillus subtilis*	110	L(+)	Inhibitory; competitive with pyruvate
Lactate analogs	*Bacillus subtilis*	110	L(+)	Slightly inhibitory at 7.7 mM in 0.05 M phosphate, pH 7.2
ATP	*Lactobacillus casei*	40	L(+) (FDP)	Strongly inhibitory
	Lactobacillus plantarum	64	D(−), L(+)	Inhibitory
	Lactobacillus (various species)	35	D(−), L(+)	Not inhibitory
	Leuconostoc lactis	38	D(−)	50% inhibition at 1.0 mM (pH 5.4)
	Streptococcus cremoris	52	L(+) (FDP)	Inhibitory in triethanolamine buffer, pH 8.0
		53		Inhibitory; competitive with NADH
	Streptococcus mutans	6	L(+) (FDP)	Inhibitory
		108		Not inhibitory
	Pediococcus pentosaceus	39	D(−), L(+)	Inhibitory; competitive with NADH for both LDHs at pH 5.4 and for D(−)-LDH at pH 7.0
	Escherichia coli	91	D(−)	Inhibitory
	Staphylococcus epidermidis	41	L(+) (FDP)	Slight inhibition
	Acholeplasma laidlawii	70	L(+) (FDP)	Inhibitory; competitive with NADH
	Bacillus caldolyticus	97		Not inhibitory
	Butyribacterium rettgeri	99, 102	D(−)	50% inhibition at 4.0 mM
	Selenomonas ruminantium	96	L(+)	Inhibitory
	Actinomyces viscosus	5	L(+) (FDP)	Inhibitory; at Hill value of 2.24, inhibition decreased as phosphate concentration increased
	Rothia dentocariosa	21	L(+) (FDP)	Inhibitory at 1.0 mM
Adenosine diphosphate	*Lactobacillus casei*	40	L(+) (FDP)	Strongly inhibitory
	Lactobacillus plantarum	64	D(−), L(+)	Inhibitory
	Lactobacillus (various species)	35	D(−), L(+)	Not inhibitory
	Leuconostoc lactis	38	D(−)	50% inhibition at 2.5 mM (pH 5.4)
	Streptococcus mutans	6	L(+) (FDP)	Inhibitory
		108	L(+)	Not inhibitory at 0.33 mM

TABLE 6—*Continued*

Substance	Taxon	Reference	Type of LDH	Effect on enzyme
	Pediococcus pentosaceus	39	D(−), L(+)	Slight inhibition
	Staphylococcus epidermidis	41	L(+) (FDP)	Inhibitory; competitive with NADH
	Bacillus caldolyticus	97		Inhibitory
	Selenomonas ruminantium	96	L(+)	Inhibitory, but less than ATP
	Lactobacillus casei	40	L(+) (FDP)	Slight inhibition
NAD	*Leuconostoc lactis*	38	D(−)	50% inhibition at 2.9 mM
	Pediococcus pentosaceus	39	D(−), L(+)	Slight inhibition
	Selinomonas ruminantium	96	L(+)	Slight inhibition

[a] Adenosine monophosphate has little effect (35, 38, 41, 64, 96), except for *Bacillus caldolyticus* (97).

S. mutans (6, 107), *L. casei* (17), and *A. laidlawii* (70). At high concentrations some phosphorylated metabolic intermediates are inhibitory to the nLDH of *L. casei* (40). However, the inhibition by adenosine triphosphate (ATP) and other adenosine phosphates is not linked to FDP activation, as ATP is competitive with NADH (39, 41, 53, 70).

The stimulatory effect of FDP is linked to pH (46), and at pH 4.5 FDP has no effect on the activity of the nLDH of *L. casei*. However, pH 4.5 is probably below any physiological pH. The nLDH's of a number of species of streptococci have been tested under the same conditions at pH 7.0 and 5.5. The effects of FDP on the LDHs of different species are varied (30, 32, 33). Similar variations in FDP activation of LDHs are found in the lactobacilli (46) and some streptococci (105, 107).

S. thermophilus (31) has L(+)-nLDH's which are apparently not affected by FDP in vitro, and two other strains of streptococci are reported to possess similar LDHs. Of these LDHs, that of *S. bovis* (106) was tested without purification under only one set of conditions, and it is difficult to be sure that under other conditions FDP would affect the enzyme. The other nLDH was found in a strain of *S. mutans* (108). The assays were performed in 67 mM phosphate, and this high concentration makes it difficult to interpret the observations.

The level of FDP used for various assays has ranged from 3.0 mM (46) downward. The level of FDP required is influenced by the choice and concentration of buffer, pH, level of pyruvate, and species of bacterium. When a wide range of streptococci and *L. casei* have been used, the assay conditions given for the group N streptococci (30) have been satisfactory. The FDP level (0.5 mM) is lower than many workers choose but 1.0 mM has been found to be slightly inhibitory to some LDHs (Garvie, unpublished data), particularly those of *S. lactis* and *S. cremoris*, in which even 0.5 mM is slightly inhibitory under some conditions (30). The nLDH of *A. laidlawii* is activated by as little as 1 μM FDP, and even this level changes the affinity of the enzyme for NADH and pyruvate (70).

The FDP-activated nLDH's are particularly sensitive, and they can be inhibited by many commonly used buffering compounds (10, 40, 48); phosphate, citrate, maleate, imidazole, and histidine all inhibit the nLDH's of *S. lactis* and *L. casei*. *A. viscosus* nLDH (5) is affected differently and is inhibited by Tris-glycine and also by sodium acetate, a buffer which does not affect the nLDH's of the lactic acid bacteria. Some nLDH's not affected by FDP can be inhibited by buffers (35).

There is little information on protecting enzymes against inhibition and inactivation. The nLDH of *E. coli* is protected by pyruvate or NADH against the effect of arsenite but not by mercaptoethanol or dithiothreitol (90). There is no lactate inhibition of this enzyme, but, as it is irreversible, lactate may not bind (91). Lactate (0.44 M) does not inhibit the L(+)-nLDH of *S. epidermidis* (43) (another irreversible LDH), nor is lactate inhibitory to the reversible nLDH of *Leuconostoc lactis* (38). Inhibition by pyruvate is seldom reported, but the nLDH of *S. aureus* was inhibited by high concentrations of pyruvate (24).

Inactivation of the nLDH of *B. subtilis* by dilution was not prevented by 0.01 M pyruvate, 1 mM NADH, 1 mM ATP, 1 mM adenosine diphosphate, 1 mM ethylenediaminetetraacetate, or 1 mM NAD (110). The nLDH of *S. epidermidis* (43) can be reactivated after dialysis in 0.02 M phosphate (pH 6.2) by the addition of FDP. During dialysis the enzyme dissociates into monomers, but the addition of FDP restores the tetrameric form. This enzyme appears to behave differently from the enzymes of various species of streptococci because in this laboratory the addition of FDP has not been found to reactivate streptococcal nLDH's which have lost activity.

Specificity. The nLDH's are highly specific and can only use NADH as coenzyme. Some enzymes use pyruvate analogs but with low affinity (13, 35, 39, 46); others do not use them

TABLE 7. *Effect of metal ions and sulfhydryl-binding compounds on some nLDH's*

Taxon	Reference	Type of LDH	Metals		Sulfhydryl-binding compounds	
			Inhibition	No inhibition	Inhibition	No inhibition
Lactobacillus						
L. acidophilus	45	L(+)			Iodoacetamide (slight)	*p*-Hydroxymercuribenzoate
L. casei	45, 46	L(+) (FDP)	Cd^{2+}, at high concentration	Mn^{2+},[a] Co^{2+},[a] Cd^{2+},[a] Ca^{2+}[a]	Iodoacetamide (slight)	*p*-Hydroxymercuribenzoate
	40	L(+) (FDP)	Hg^{2+}, Cu^{2+}	Mn^{2+},[a] Ca^{2+}, Co^{2+}, Mg^{2+}		*p*-Hydroxymercuribenzoate
	17	L(+) (FDP)		Mn^{2+},[a] Mg^{2+}		
	16	L(+) (FDP)		Mn^{2+}[a]		
	48	L(+) (FDP)		Mg^{2+}, Mn^{2+},[a] Co^{2+},[a] Cd^{2+},[a] Cu^{2+},[a] Ni^{2+}[a]		
L. curvatus	45, 46	L(+) (FDP)		Mn^{2+},[a] Co^{2+},[a] Cd^{2+},[a] Ca^{2+} (slight)[a]		*p*-Hydroxymercuribenzoate, iodoacetamide
Leuconostoc lactis	38	D(−)	Hg^{2+}, Cu^{2+}	Ca^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}	*p*-Chloromercuribenzoate (50% inhibition at 0.4 mM)	Iodoacetamide
	49	D(−)			*p*-Chloromercuribenzoate (complete at 1 mM)	
Streptococcus						
S. faecalis	10	L(+) (FDP)		Mn^{2+}[a]		
S. cremoris	20	L(+) (FDP)			*p*-Chloromercuribenzoate	Iodoacetamide, iodoacetate
S. lactis	10	L(+) (FDP)		Mn^{2+}, Na_2SO_4, KCl		
S. mutans	27	L(+) (FDP)		$MgCl_2$, $MnCl_2$, $CaCl_2$, $ZnCl_2$		
Pediococcus pentosaceus	39	D(−)	$HgCl_2$, $CuSO_4$	$CaCl_2$, $CoCl_2$, $MgCl_2$, $MnCl_2$	*p*-Hydroxymercuribenzoate (50% inhibition at 0.26 mM, pH 5.4)	Iodoacetamide
		L(+)		$CaCl_2$, $CoCl_2$, $MgCl_2$, $MnCl_2$	*p*-Hydroxymercuribenzoate (50% inhibition at 0.12 mM, pH 7.4)	
		L(+)			*p*-Hydroxymercuribenzoate (50% inhibition at 0.6 mM, pH 5.4)	
Escherichia coli	90	D(−)	Sodium arsenite		*p*-Hydroxymercuribenzoate, reversible; iodoacetate and iodoacetamide, both at 4 mM	
Bacillus						
B. caldolyticus	97				*p*-Chloromercuribenzoate	
B. subtilis	110	L(+)	Many metal ions, particularly Ag, overcome by cysteine, KCN, or mercaptoethanol	$MgSO_4$, NaCl, $(NH_4)_2SO_4$	*p*-Chloromercuribenzoate	Iodoacetamide
Butyribacterium rettgeri	102	D(−)	$MgCl_2$		*p*-Chloromercuriphenyl sulfonic acid	
Mammal	77	L(+)	I^-, Ag^+, Hg^{2+}		*p*-Mercuribenzoate; 2,4-dinitrofluorobenzene and other sulfhydryl-binding compounds	

[a] Unequivocal stimulation under some conditions.

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Butyribacterium rettgeri	102	D(−)	$MgCl_2$			
Mammal	77	L(+)	I^-, Ag^+, Hg^{2+}	overcome by cysteine, KCN, or mercaptoethanol		*p*-Chloromercuriphenyl sulfonic acid *p*-Mercuribenzoate; 2,4-dinitrofluorobenzene and other sulfhydryl-binding compounds

(23). There are no reports of lactate analogs being oxidized, but the K_m values of the reversible nLDH's for lactate are high.

Pyruvate analogs have an interesting effect on the kinetics of some nLDH's. α-Ketobutyrate replaces pyruvate at the allosteric binding site but not at the substrate binding site of *L. casei* (46), and oxamate changes the pyruvate saturation curve (40).

Stability of nLDH's. There is little information regarding the stability of nLDH's, probably because few workers have found any problems. Usually the enzymes have been kept cold; however, an nLDH of *S. mutans* was found to lose activity at 4°C, but to be stable at 20°C (108). The LDH of *A. viscosus* is reported to behave in a similar manner (5).

Some enzymes have been reported to lose activity if exposed to certain conditions. The nLDH of *S. mutans* has been reported to be unstable on dialysis against 0.04 M phosphate or Tris-chloride buffer (108). The concentration of phosphate used was low, and it could well be that 0.1 M phosphate or a low pH would stabilize the enzyme, because *S. mutans* FDP-activated nLDH's are favored by a low pH. A pH below neutral is necessary with several LDHs; that of *S. cremoris* was stable at pH 6.0 to 6.5 but lost activity at alkaline pH (53, 67). The D(−)-nLDH of *B. rettgeri* loses activity at pH values above 7.0 at −20°C, and reducing agents do not stabilize it. It can be stored in phosphate buffer at pH 6.2 (102). *L. casei* nLDH was found to be more stable than the nLDH of *L. curvatus* under a variety of conditions. The latter enzyme lost activity at pH 7.5 (46).

Storage conditions are important to the stability of the enzymes. *S. cremoris* nLDH was stored in sodium phosphate (pH 7.0) (I = 0.1) containing 35% $(NH_4)_2SO_4$ for some studies (52). The enzyme then had to be dialyzed before being used; this is not a particularly convenient technique, and it has not been used with any other nLDH. The nLDH of *S. ruminantium* can be stored deep frozen (−60°C) and is stable at room temperature during a working day (96). The enzyme of *A. aerogenes* is unusual because the activity was increased by exposure to KCl at concentrations up to 0.2 M. Other compounds had similar but smaller effects (72).

Many of the FDP-activated nLDH's lose activity if phosphate or FDP is not present in a high enough concentration (33, 106); once these nLDH's have lost activity, no one has found conditions to recover it. The nLDH of *B. subtilis* is inactivated by dilution, but activity was regained by precipitating with $(NH_4)_2SO_4$ at pH 7.2 to 7.5 and redissolving in buffer at the same pH (110). Might this technique work with other LDHs?

Heat stability. Bacterial nLDH's vary greatly in their sensitivities to heat. The optimum temperature of activity has not been investigated closely. The enzymes are active at 25°C, and 30°C has been selected for assaying the enzymes in only a few studies. However, the optimum temperature for the nLDH of *B. caldolyticus*, a thermophilic organism from hot springs, is 55 to 60°C and apparently varies with the composition of the medium in which the organism is grown (97). The heat stability of the enzyme was found to be complex. Information on the heat stability of various nLDH's is shown in Table 8. The methods used to collect these data were not identical, and therefore it is difficult to compare the effects of heat on the various nLDH's. It is clear that there is considerable variation among the different types of nLDH's (13, 35) and among the nLDH's of different species (35, 45).

Molecular weights of nLDH's. Various techniques have been used to determine molecular weights, but all have arrived at similar values. There is general agreement that the L(+)-nLDH's have molecular weights of about 140,000 (6, 20, 39, 40, 41, 43, 45, 52, 111), but the molecular weight of *L. acidophilus* nLDH was only 120,000 (35). The nLDH molecule is a tetramer (20, 39, 40, 43, 45). It may be split into subunits in different ways; the LDH of *S. cremoris* is irreversibly denatured into units of 72,000 daltons at pH 9.0 (52). The nLDH of *S. epidermidis* was found to have a molecular weight of 120,000 at its optimum pH of 5.6 but only 68,000 ± 2,000 between pH 6.5 and 7.0. At pH 8.5 or 2.0 inactive monomers are formed (molecular weight, 36,000) (41).

The L(+)-nLDH of *R. dentocariosa* is small, having a molecular weight of only 120,000 (21), whereas that of *A. viscosus* has a molecular weight of 100,000 ± 10,000 (5). These are both FDP-activated L(+)-nLDH's, but they are smaller than the similar enzymes found in the lactic acid bacteria. If FDP-activated nLDH's have a common origin, then major changes have taken place in some of the enzymes. A more likely explanation is that FDP control has arisen separately in different genera. The L(+)-nLDH of *B. subtilis* has a molecular weight of 140,000 and is a tetramer, but inactive dimers of 72,000 daltons are found (111), depending on the buffer. The D(−)-nLDH's of the lactic acid bacteria and staphylococci on the other hand have molecular weights between 70,000 and 80,000 (23, 27, 35, 38, 39, 42, 49). These enzymes are dimers (28, 38, 39, 42, 49).

The molecular weights of the D(−)-nLDH's of *E. coli* (90) and *A. aerogenes* (74) are given as

TABLE 8. *Effect of heat on the activity of some nLDH's*

Taxon	Reference	Effect of heat	Stabilizing agents
L(+)-LDHs			
Lactobacillus acidophilus	35	Active after 5 min at 50°C and 50% inactivated after 5 min at 80°C (strain 65K) Active after 5 min at 50°C and 20% inactivated after 5 min at 80°C (strain A18)	
Lactobacillus plantarum	13	94% activity retained after 3 min at 50°C	
	64	Inactivated after 3 min at 60°C	
Pediococcus pentosaceus	39	Inactivated after 3 min at 55°C	
Bacillus subtilis	111	Not inactivated after 4 h at 50° C in 0.01 M phosphate, pH 7.2	
FDP-activated L(+)-LDHs			
Lactobacillus casei	46	Relatively stable at 60°C	
	40	Inactivated after 3 min at 60 to 70°C in 0.05 M phosphate	
Lactobacillus curvatus	46	Inactivated at 60°C	Mn^{2+}; FDP
Streptococcus faecalis	100	Active after 10 min at 67°C	FDP increased heat sensitivity; NADH protected
Streptococcus cremoris	53	Inactivated after 10 min at 50°C in 0.01 M phosphate, pH 7.0	Phosphate stabilized, but this was not due to ionic conditions
Streptococcus mutans	6	Inactivated after 5 min at 45°C in absence of FDP	FDP protected against heat inactivation
Staphylococcus epidermidis	41	Inactivated after 2 min at 50°C	Protected by a mixture of FDP, NADH, and pyruvate
Actinomyces viscosus	5	Inactivated after 20 min at 65°C	Protected by ATP; sensitized by NADH; pyruvate had no effect
D(−)-LDHs			
Lactobacillus acidophilus	35	98% inactivated after 5 min at 45°C	
Lactobacillus jensenii	35	25% inactivated after 5 min at 50°C; 100% inactivated after 5 min at 80°C	
Lactobacillus leichmannii	35	50% inactivated after 5 min at 50°C; 100% inactivated after 5 min at 80°C	
Lactobacillus plantarum	13	Inactivated after 3 min at 50°C	
	64	Inactivated after 3 min at 45°C	
Lactobacillus fermentum	35	98% inactivated after 5 min at 50°C	
Leuconostoc lactis	49	98% inactivated after 5 min at 45°C; inactivated slowly at 37 and 42°C	Protected by D(−)-lactate or NAD
Leuconostoc mesenteroides	23	Stable below 40°C in phosphate, pH 7.5; stable below 35°C in Tris-chloride, pH 8.5	
	38	Inactivated at 45 to 50°C	
Pediococcus pentosaceus	39	Inactivated after 3 min at 55°C	
Staphylococcus haemolyticus	42	Inactivated after 10 min at 50°C	Protected by D(−)-lactate with NAD
Staphylococcus hominis	42	Inactivated after 10 min at 50°C	Protected by NADH with or without pyruvate
Staphylococcus warneri	42	Inactivated after 10 min at 50°C	Protected by NADH but not if pyruvate was present

112,000 and 110,000 to 140,000, respectively. These enzymes are therefore larger than the D(−)-nLDH's of the lactic acid bacteria.

Amino acid compositions. The amino acid compositions have been determined for a few bacterial LDHs, namely, those of *L. mesenteroides* (23), *E. coli* (90), *L. casei*, *L. curvatus*, *L. acidophilus* (45), *L. plantarum* (15, 45), *S. cremoris* (20, 52), and *B. subtilis* (111) (Table 9).

The results are usually given for a subunit (i.e., molecular weight of 34,000). The results for *S. cremoris* are given for a "monomer" of 70,000 daltons, so that these figures should be halved to make a comparison with the figures for the lactobacilli. However, it is pointed out that the monomer consists of two identical subunits, and each of these has only one cysteine residue (52). A low cysteine content has also been found in the *Lactobacillus* nLDH's examined (45), but not in *L. mesenteroides* (23). A low cysteine content may be linked to the insensitivity of bacterial nLDH's to thiol inhibitors. It still has to be decided whether thiol groups are essential for dehydrogenase activity, but it appears that the significance of cysteine in bacterial nLDH's is not very great.

There are considerable differences in the four *Lactobacillus* LDHs, and these differences are discussed in relationship to the evolution of bacteria (45). There are greater differences among the nLDH's of four species of one genus than among the nLDH's of six vertebrates from four different classes. Information on the structures, binding sites, and operation of bacterial nLDH's

TABLE 9. *Amino acid compositions of some bacterial LDHs and mammalian LDH*

TABLE 9. *Amino acid compositions of some bacterial LDHs and mammalian LDH*

Taxon	Reference(s)	Type of LDH	Amino acid composition																Mol wt
			Lysine	Histidine	Arginine	Asparagine	Threonine	Serine	Glutamine	Proline	Glycine	Alanine	Half-cysteine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Tryptophan	
Lactobacillus acidophilus	45	L(+)	21.5	8.2	9.7	32.5	16.8	17.9	35.2	12.8	30.4	31.3	1.2[a]	28.3	9.4	19.0	26.7	7.3	8.0	2.0	35,000
Lactobacillus plantarum	45	L(+)	22.3	3.3	10.7	40.0	14.4	21.5	26.6	12.0	31.6	32.4	1.6[a]	22.1	9.3	17.0	27.7	5.5	10.2	0.8	35,000
Lactobacillus plantarum	15	L(+)	112	10	56	156	54	81	108	62	126	145	4	82	21	84	124	52	45	0	140,000
Lactobacillus casei	45	L(+) (FDP)	24.2	5.3	8.5	39.7	17.4	18.7	28.5	13.2	24.0	34.2	1.1[a]	23.6	6.7	27.4	24.6	10.1	9.7	1.2	35,000
Lactobacillus curvatus	45	L(+) (FDP)	21.9	4.8	7.3	32.0	17.2	20.0	30.8	13.8	24.2	32.2	2.2[a]	20.9	7.8	29.3	23.8	7.7	9.7	3.1	35,000
Leuconostoc mesenteroides	23	D(−)	26	11	20	68	26	21	48	22	37	61	28	35.5	12	34	45	17	18	7	70,000[b]
Streptococcus cremoris	52	L(+) (FDP)	43	13	20	72	31	37	72	19	48	73	3	66	11	38	50	19	26	5	70,000
Escherichia coli	90	D(−)	60	29	63	105	56	54	116	40	76	91	12	76	28	59	109	40	40	7.1	100,000[b]
Bacillus subtilis	111	L(+)	23	9	6	39	17	18	33	11	31	32	4	29	6	20	25	13	13	2	35,000[c]
Bovine (M)	15, 73	L(+)	103	33	42	127	48	87	121	51	100	78	26	115	32	91	136	29	29	22	140,000[b]

[a] Cysteic acid.
[b] Molecular weight of 1 mol.
[c] Molecular weight of one subunit.

is far too slight for any conclusions to be drawn at the present time. The differences in amino acid compositions can only be noted and perhaps understood at some time in the future.

Tryptic digests of the L(+)-nLDH's of four strains of lactobacilli showed different fingerprint patterns after chromatography (45). Only one peptide was common to all of the enzymes, and it was comparable to the "arg 6" peptide of mammalian nLDH. Amino acid sequencing of this peptide showed remarkable similarities among the four bacterial and mammalian nLDH's. The bacterial enzymes were even more closely allied to lobster tail nLDH, for in these enzymes the "essential cysteine" of the arg 6 peptide is replaced by threonine. The arg 6 peptide is the substrate binding site in mammalian and lobster tail nLDH's, and the similarities shown by the bacterial nLDH's suggest that the peptide studied was the substrate binding site. Final confirmation of this fact is still awaited.

Comparisons with mammalian LDHs. Mammalian nLDH's have been studied in great detail, but the same attention has not been given to any bacterial LDH. To microbiologists the similarities among the nLDH's of different species of mammals are strange because such close similarities between nLDH's in two species of procaryotes would suggest that the classification should be checked and that the two species may in fact be one. D(−)-nLDH and iLDH's specific for lactate are important in many bacteria but unknown in mammals. Similarly, the interesting group of FDP-activated nLDH's has no parallel in higher animals. Therefore, we should try to compare the L(+)-nLDH's in bacteria and mammals. Like the bacterial nLDH's, the mammalian nLDH's are highly specific; 2,4-diketo acids are reduced only slowly, and NAD is not replaced by nicotinamide adenine dinucleotide phosphate (4, 77). Both lactate and pyruvate act as inhibitors under some conditions.

No bacteria have yet been shown conclusively to possess two separate nLDH's which catalyze the formation of the same isomer of lactate. Isoenzymes such as those found between the H and M types in mammals are not found in bacteria.

Some information about mammalian LDHs is included in Tables 2 through 5, 7, and 9 so that a comparison can be made with bacterial enzymes. The optimum pH (Table 3) of many bacterial LDHs is lower than the optimum pH of mammalian LDHs. The K_m values show a marked difference, particularly with the values for lactate and NAD (Table 5). These values reflect the reversibility of the mammalian LDHs, compared with the irreversibility of the

bacterial LDHs. The reversibility is linked to function, for although lactate is an end product discarded by bacteria, lactate formed in vertebrates is reconverted to pyruvate again by the M-type LDH.

Mammalian nLDH has a high cysteine content, whereas bacterial nLDH cysteine content is low. The significance of these differences is discussed elsewhere for the lactobacilli (45) and streptococci (20). These differences are reflected in different responses to a number of inhibitors (Tables 5 and 6).

ATP, which is often an inhibitor of bacterial nLDH's, is not mentioned in the literature on mammalian nLDH's. There is some inhibition of mammalian nLDH's with acetate, maleate, and citrate (77), but the effects are smaller than with the bacterial nLDH's where such inhibition is known to occur. Oxamate is an inhibitor of both pyruvate reduction and lactate oxidation (77). *p*-Mercuribenzoate inhibits slowly but can be reversed by cysteine and glutathione; Hg and Ag salts also inactivate, but iodoacetate and *N*-ethylmaleimide do not.

The differences between the vertebrate nLDH's and the bacterial nLDH's may not be any greater than those found among bacterial nLDH's. The question which awaits an answer is why has the structure been conserved in so many vertebrates but diversified (or evolved separately) among the procaryotes. Eucaryote LDHs are protected inside the organisms, so that evolutionary pressures which cause changes are likely to be minimal. Procaryote LDHs may have evolved several times; even so, the diversity of LDHs within a genus suggests that evolutionary pressures causing modifications are strong. These may affect terminal bacterial nLDH's easily and cause modifications in structures and properties. The advantages gained by any particular modification are not understood. Further knowledge of the properties of the enzymes might clarify the reasons for the differences.

iLDH's

nLDH's, which form lactate from pyruvate, are terminal enzymes, and a reasonable indication of their properties can be obtained from cell extracts without purification. Even when iLDH's are also present, it is possible to assay the nLDH's by using lactate as the substrate; NAD operates with only one type of LDH. The iLDH's are not terminal enzymes. Work without purification may measure the activity of the iLDH plus the activities of other electron transport proteins. Only a few iLDH's have been studied after even partial purification.

The species in which iLDH's have been examined are shown in Table 1. Some of the enzymes have been purified, and many of their properties are known [e.g., *E. coli* D(−)-iLDH], but in several instances only the existence of iLDH's is known because they have been detected in crude cell extracts (e.g., *Acetobacter peroxydans* [12] and *S. lactis* and *S. cremoris* [2]).

iLDH's are known in a variety of bacterial genera, but their functions are different in different genera. All iLDH's convert lactate to pyruvate, and no evidence of a reverse reaction has been found. The iLDH's can be divided into three broad groups. The iLDH's belonging to first group are found in *E. coli* and *A. aerogenes*; the D(−)-iLDH is constitutive (58, 71), particulate, and linked to electron transport (22, 59). Earlier studies have extracted both D(−)- and L(+)-iLDH's (44, 88). Enzymes of a similar sort are probably found in other species of the genera in the *Enterobacteriaceae*. The L(+)-iLDH in *E. coli* (and *A. aerogenes*) is also particulate (44, 58) but has not been studied much. The iLDH's in the second group enable bacteria to use lactate as a source of carbon. These enzymes appear to be soluble, although particulate enzymes have been described (12). The iLDHs in the third group occur in the lactic acid bacteria, where they are soluble and are known in a number of species (27, 34, 55, 65, 83). The concentrations of the iLDH's in the cells are usually low, and they are probably inert when a cell is actively forming lactate. When nutrients are in short supply, the iLDH's may scavenge some of the lactate and reconvert it to pyruvate, which the cell can then use. Only the enzymes in *L. plantarum* (83) and *L. casei* (65) have been studied in any detail. Properties of these enzymes are shown in Tables 10 through 15.

D(−)-iLDH of *Escherichia coli*. Although it was demonstrated in 1959 that *E. coli* had both a D(−)-iLDH and an L(+)-iLDH (44), it was not until 1973 that the D(−)-iLDH was purified and some of its properties were described (22, 59). The amount of D(−)-iLDH per cell remains constant irrespective of the carbon source in the medium (44, 58, 71), whereas the L(+)-iLDH is induced by aerobic growth on lactate and absent from glucose-grown cells.

Glyoxalate (59) and lactate aerobically (22, 89) have been used for the large-scale cultivation required for the purification of the D(−)-iLDH. Cells are broken either by homogenization or by treatment with lysozyme. Whole cells can be kept at −90°C for at least 3 months (22, 59).

The properties of the purified D(−)-iLDH are shown in Table 10. Two different methods of purification were used (22, 59), but both used detergent. The enzyme has now been obtained free of detergent (89); 1 mM β-mercaptoethanol

TABLE 10. *Some properties of the* D*(−)-iLDH of E. coli ML308-225 ($i^-z^-y^+a^+$)*

Property[a]	Value and/or comment	Reference(s)
Specific activity[b]	460 U (with DCPIP), 75.4 U (with MTT)	59
	81.5 U (with MTT)	22
K_m[c]	D(−)-Lactate, 1.4 mM; L(+)-lactate, 22 mM	59
	2.2 mM in membrane	22
	0.6 mM when purified	22
	0.8 to 0.5 mM[d]	58
Stability	Two weeks in ethylene glycol at −90°C after purification	22
Specificity	Only D(−)-lactate; NAD does not affect activity	22, 59
Molecular weight	73,000 to 75,000; 71,000 ± 3,000	22, 59
	180,000 to 240,000 without detergent	89
Optimum pH	8 to 9 for purified LDH; 7.0 in membrane, which was raised to more than 9 when cyanide was present	22
	7.8 to 8.7 for purified LDH; 7.2 to 8.0 before detergent treatment, but 6.2 to 6.8 in vesicles	59
Prosthetic group[e]	FAD (1 mol/mol of LDH), but FAD does not reactivate the apoenzyme. The FAD is not covalently bound, as it is extractable with trichloroacetic acid	22, 59
Product inhibitors[f]	Pyruvate	59
	Oxamate and oxalate (competitive)	22, 59
Electrophoresis	LDH can be demonstrated after electrophoresis at pH 9.5 with lactate, Nitro Blue Tetrazolium, and phenazinemethosulfate	59

[a] Two sets of assay conditions were used. The first set was as follows (59): 0.1 M phosphate buffer, pH 7.8; 30 μg of 2-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) per ml; 60 μg of phenazinemethosulfate per ml; 10 mM D(−)-lactate; 25°C; 0.04 mg of dichlorophenol indophenol (DCPIP) per ml was also used. The second set of assay conditions was as follows (22): 0.08 M Tris-chloride buffer, pH 8.0; 60 μg of MTT per ml; 120 μg of phenazinemethosulfate per ml; 10 mM D(−)-lactate; 23°C; 0.02 mg of DCPIP per ml was also used.

[b] One unit of activity equals 1 μmol of MTT reduced per min.

[c] The K_m varies with the presence or absence of detergent and is lowest with the aggregated LDH and highest in the membrane (2.2 to 0.49 mM) (89).

[d] For assay conditions, see Table 11.

[e] FAD, Flavin adenine dinucleotide.

[f] Sulfhydryl agents (0.01 M) do not inhibit, nor do Mg^{2+}, Ca^{2+}, Na^+, and K^+. Arsenate inhibits slightly; cyanide inhibits activity in the membrane at alkaline pH's but stimulates it at a neutral pH. Pure enzyme was inhibited 50% by 1.5 mM cyanide (pH 9.3).

is used to stabilize the enzyme during purification.

In association with detergent the enzyme is a monomer, but aggregates of two to three units form in the absence of detergent (89). Detergent activates the aggregated D(−)-iLDH but does not dissociate it into a monomer. Detergent would not be present in vivo and the D(−)-iLDH has a greater affinity for phospholipids, which also activate it, than for detergent. The effects of detergent and phospholipids on the aggregation and activity of the enzyme are not yet understood. It is thought that the enzyme is a monomer in vivo.

Comparisons of the specific activities of iLDH's are complicated because there is no universal definition of a unit of activity. The natural hydrogen acceptor is not used in assays, and the reduction of a dye is used to monitor the reaction. The most commonly used dye is dichlorophenol indophenol but in the case of *E. coli* the reduction of phenazinemethosulfate is coupled to the reduction of 2-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide. Dichlorophenol indophenol and methylene blue also act as hydrogen acceptors. The units of enzyme activity which have been used for different iLDH's are shown in Table 11.

iLDH's of other *Enterobacteriaceae*. *A. aerogenes* also has particulate iLDH's. Like the D(−)-iLDH of *E. coli*, that of *A. aerogenes* can use dichlorophenol indophenol or phenazinemethosulfate as a hydrogen acceptor. The latter cannot use ferricyanide or cytochrome *c* (71), whereas the former can (58); however, *E. coli* can use cytochrome *c* only if phenazinemethosulfate is also present. *Serratia, Aerobacter cloacae, Proteus vulgaris, Escherichia freundii, Klebsiella* sp., and *Hafnia* sp. have iLDH's which are induced under the same conditions as the iLDH's of *A. aerogenes*, but in *Salmonella typhimurium* no iLDH's are formed under anaerobic conditions (71).

The L(+)-iLDH's in the *Enterobacteriaceae*

TABLE 11. *Experimental conditions used for the assay of iLDH's*[a]

Taxon	Reference	Type of LDH[b]	Temp (°C)	Buffer	Buffer concn (M)	Lactate concn (mM)	H^+ acceptor(s)[c]	Concn of acceptor	Units of enzyme activity
Aerobacter aerogenes	71	D(−) + L(+)	25	Phosphate	0.125	10.0	DCPIP	0.045 mM	
Escherichia coli	58	D(−) + L(+)		Sodium phosphate[d]	0.066	13.3	DCPIP	0.038 mM	1 μmol of substrate oxidized per h
							$K_3Fe(CN)_6$	0.66 mM	1 μmol of substrate oxidized per h
							cytochrome *c*	0.08 mM	1 μmol of substrate oxidized per h
							PMS	0.67 mg/ml	1 μmol of substrate oxidized per h
Pseudomonas aeruginosa	57	D(−) + L(+)	30	Sodium-potassium phosphate[e]	0.02	2.0	DCPIP	0.2 mM	1 μmol of substrate oxidized per min
							PMS	1.0 mM	1 μmol of substrate oxidized per min
							$K_3Fe(CN)_6$	2.0 mM	1 μmol of substrate oxidized per min
							O_2	0.24 mM	1 μmol of substrate oxidized per min
Propionibacterium pentosaceum	66	D(−) + L(+)	25-27	Potassium phosphate	0.02	16.6	DCPIP	0.03 mM	1 μeq of DCPIP reduced per min
							1,2-naphthaquinone sulfonate	0.03 mM	
Staphylococcus aureus	80	L(+)		Phosphate	0.01	Varied	DCPIP	0.002%	
Butyribacterium rettgeri	101	D(−) + L(+)		Potassium phosphate	0.1	10.0	$K_3Fe(CN)_6$	0.8 mM	1 μmol of $K_3Fe(CN)_6$ reduced per min
	104						DCPIP	0.05 mM	1 μmol of DCPIP reduced per min
Lactobacillus casei	65	D(−) and L(+)		Phosphate	0.15	8.0	DCPIP	0.08 mM	ΔOD_{600} of 0.1/min[f]
Lactobacillus plantarum	83	D(−)	28	Tris-maleate	0.02	33	DCPIP	0.033 mM	1 μmol of substrate oxidized per min
		L(+)	28	Tris-maleate	0.02	33	DCPIP	0.033 mM	1 μmol of substrate oxidized per min
Lactic acid bacteria (various species)	18	D(−) and L(+)	30	Tris-maleate	0.02	ca. 15.0	DCPIP	8.66 μg/ml	ΔOD_{578} of 0.001/min[g]
Leuconostoc (various species)	27	D(−) and L(+)	25	Tris-maleate	0.1	100	DCPIP	5.2 μg/ml	
Streptococcus faecium	60	D(−)		Phosphate		16.6	DCPIP	0.033 mM	1 nmol of DCPIP reduced per min
Streptococcus lactis/S. cremoris	2	D(−) and L(+)		Triethanolamine	0.083	33.3	DCPIP	0.033 mM	
Pediococcus pentosaceum	55	D(−)		Phosphate	0.25	Varied	DCPIP	0.1 mM	

[a] The LDHs of the following species were also assayed in a Warburg apparatus: *Acetobacter peroxydans* (12) (O_2 was used for particulate enzymes, but methylene blue or phenazinemethosulfate was used for soluble enzymes), *Escherichia coli* (44), and *Propionibacterium pentosaceum* (66). Thunberg tubes were used for *E. coli* (88) and *Pseudomonas natriegens* (95); methylene blue was added as an H^+ acceptor.

[b] D(−) + L(+), Activities were not separated (tested together); D(−) and L(+), activities were separated (assayed individually).

[c] DCPIP, Dichlorophenol indophenol; PMS, phenazinemethosulfate.

[d] KCN (0.67 mM) was included in the assays.

[e] CN^- (1.0 mM) was included in the assays, except when O_2 was present.

[f] ΔOD_{600}, Change in optical density at 600 nm.

[g] ΔOD_{578}, Change in optical density at 578 nm.

TABLE 12. *Electron acceptors used by iLDH's*[a]

Taxon	Reference	LDH type	Cytochrome *c*	Cytochrome *c* + PMS	$K_3Fe(CN)_6$	DCPIP	MB	PMS
Escherichia coli	58	D(−)	−	+	+	+		
		L(+)	−	+	+	+		
Aerobacter aerogenes	71	D(−) + L(+)[b]	−		−	+		+
Pseudomonas aeruginosa	57	D(−)	+		+	+		+
		L(+)	+		−	+		+
Pseudomonas natriegens[c]	95	D(−) + L(+)			−		+	
Acetobacter peroxydans[d]	12	D(−) + L(+)						
Butyribacterium rettgeri	104	D(−) + L(+)			+	+	−	
Propionibacterium pentosaceum[e]	66	D(−) + L(+)				+	+	+
Lactobacillus casei	65	D(−)				+	+	
		L(+)				+	−	
Lactobacillus plantarum	83	D(−) + L(+)	−		−	+		
Pediococcus pentosaceus	55	D(−)	−		−	+		

[a] PMS, Phenazinemethosulfate; DCPIP, dichlorophenol indophenol; MB, methylene blue.
[b] D(−) and L(+) activities were not separated.
[c] Dyes with plus potentials, but not $K_3Fe(CN)_6$.
[d] There are various electron acceptors, with E_0 greater than +0.01.
[e] Other H^+ acceptors were tested but were less efficient.

TABLE 13. *Optimum pH of reaction of some iLDH's*

Taxon	Reference	LDH type[a]	Optimum pH
Pseudomonas natriegens	95	D(−) + L(+)	9.0
Butyribacterium rettgeri	104	D(−) + L(+)	6.2
Propionibacterium pentosaceum	66	D(−) + L(+)	7.7
Lactobacillus plantarum	83	D(−)	6.7
		L(+)	6.0
Lactobacillus plantarum	18	D(−) and L(+)	6.4
Lactobacillus casei	85	D(−)	6.3
		L(+)	5.6
Lactobacillus brevis	18	D(−) and L(+)	5.8
Leuconostoc mesenteroides	18	D(−) and L(+)	6.2

[a] D(−) + L(+), Both activities were tested together; D(−) and L(+), activities were tested individually.

natriegens (95); methylene blue was added as an H^+ acceptor.
[b] D(−) + L(+), Activities were not separated (tested together); D(−) and L(+), activities were separated (assayed individually).
[c] DCPIP, Dichlorophenol indophenol; PMS, phenazinemethosulfate.
[d] KCN (0.67 mM) was included in the assays.
[e] CN^- (1.0 mM) was included in the assays, except when O_2 was present.
[f] ΔOD_{600}, Change in optical density at 600 nm.
[g] ΔOD_{578}, Change in optical density at 578 nm.

have been studied only superficially. In all of the strains examined, these enzymes are induced (aerobically) by DL-lactate, but in *P. vulgaris*, *Hafnia* sp., and *S. typhimurium* they are also induced by glucose, which is, however, less effective than lactate (71).

iLDH's of other bacteria (excluding the *Lactobacilliaceae*). *Pseudomonas aeruginosa* is reported to have particulate D(−)- and L(+)-i-LDH's linked to electron transfer. In the strain examined both enzymes were induced by lactate in the growth medium (57). D(−)- and L(+)-lactate oxidation rates are different with different electron acceptors, so that it is thought that there are two separate enzymes. These iLDH's are believed to be located in the membrane of the cell. The enzymes in *P. aeruginosa* have not been separated or purified, so that more work is necessary before the roles of these enzymes are understood.

S. aureus has also been shown to link LDH activity to electron transport (80). Membrane vesicles convert L(+)-lactate to pyruvate, and dichlorophenol indophenol acts as a hydrogen acceptor. The iLDH is known only from work on membrane vesicles and appears to be induced when cells are grown on glucose.

The iLDH's of another pseudomonad have been reported (95). However, it is unlikely that the organism, *Pseudomonas natriegens*, should be called a pseudomonad because it ferments sugars. It has been tentatively reclassified as a

TABLE 14. *K_m values estimated for iLDH's*

Taxon	Reference	LDH type	K_m (mM)	Buffer			Temp (°C)
				Composition	Concn (M)	pH	
Escherichia coli[a]	58	L(+)	0.07–0.021	Phosphate	0.066	7.5	
Staphylococcus aureus	80	L(+)	0.2–0.46	Phosphate	0.01	7.3	
Propionibacterium pentosaceum	66	D(−)	0.0516[b]	Phosphate	0.25	7.3	26
Lactobacillus casei	65	D(−)	0.6	Phosphate	0.15	5.9	
		L(+)	14.0	Phosphate	0.15	5.9	
Lactobacillus plantarum	83	D(−)	2.3	Tris-maleate	0.2	6.7	
		L(+)	16.0	Tris-maleate	0.2	6.0	
Lactobacillus plantarum	18	D(−)	6.0, 4.8	Tris-maleate	0.02	6.4	
		L(+)	25.0, 12.5	Tris-maleate	0.02	6.4	
Lactobacillus brevis	18	D(−)	31.2	Tris-maleate	0.02	5.8	
		L(+)	18.5	Tris-maleate	0.02	5.8	
Leuconostoc mesenteroides	18	D(−)	18.1	Tris-maleate	0.02	6.2	
		L(+)	15.4	Tris-maleate	0.02	6.2	
Leuconostoc (various species)	78	D(−)	6.6–9.9	Tris-maleate	0.1	6.3	
Gas-forming lactobacilli	30	L(+)	66.0	Tris-maleate	0.1	6.3	
Pediococcus pentosaceus	55	D(−)	1.5	Phosphate	0.25	6.5	

[a] The K_m varied with the carbon source in the growth medium.
[b] Without NH_4^+.

TABLE 15. *Inhibitors of the reactions of some iLDH's*

Taxon	Reference(s)	LDH type[a]	Inhibitor(s)[b]
Reactions inhibited			
Escherichia coli	58	L(+)	Antimycin A, sodium amytol, and 2-*n*-nonylhydroxyquinoline-*N*-oxide, but all at high concentrations
Aerobacter aerogenes	71	L(+)	1 mM glyoxalate (70%); 1 mM oxalate had no effect
		D(−)	1 mM glyoxalate (60%), 1 mM oxalate (70%)
Pseudomonas natriegens	95	D(−) + L(+)	Atabrine, *p*-CMB, and substances which affect electron transport
Butyribacterium rettgeri	104	D(−) + L(+)	Atabrine at high concentrations
Propionibacterium pentosaceum	66	D(−) + L(+)	Pyruvate, oxalate, sulfhydryl reagents, heavy metals, narcotics, flavin antagonists
Lactobacillus casei	65	L(+)	Atabrine
Lactobacillus plantarum	82, 83	D(−) and L(+)	Atabrine (reversed by FMN or FAD), quinine sulfate (not reversed by FMN or FAD), menadione, oxalate, oxamate
Pediococcus pentosaceus	55	D(−)	Oxalate, fluorolactate (without HCN), Zn^{2+}, Mo^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+}, Fe^{3+}
Reactions not affected			
Lactobacillus casei	65	D(−)	Atabrine
Lactobacillus plantarum	82	D(−) and L(+)	*p*-CMB, NAD
Pediococcus pentosaceus	55	D(−)	Atabrine, *p*-CMB, iodoacetate, *d*-1-mandelic acid, fluoropyruvate, Co^{2+}, and Hg^{2+} (reversed by high concentrations of cysteine and histidine)

[a] D(−) + L(+), Both activities were tested together; D(−) and L(+), both activities were affected, but they were tested individually.
[b] *p*-CMB, *p*-Chloromercuribenzoate; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide.

Beneckea species (7). The enzymes were reported to be located in the cell membrane and to contain a thiol group because enzyme activity was inhibited by *para*-dichloromercuribenzoate, an unusual property for an iLDH. Although two separate iLDH's were indicated, because the activities with D(−)- and L(+)-lactate were affected differently by heat, both activities were

located in a single enzyme band after electrophoresis. An understanding of the terminal metabolism of *P. natriegens* will be achieved only by further experimentation. Particulate D(−)- and L(+)-iLDH's have been reported from *A. peroxydans*; soluble enzymes were also present, and whether these were different than the particulate enzymes was not established (12). None of these iLDH's was purified, and the effects of inhibitors and electron carriers on crude preparations were examined.

Bacteria which can grow with lactate as a source of carbon (*B. rettgeri* [104] and *P. pentosaceum* [66]) have soluble iLDH's. In *B. rettgeri* the fermentation of lactate requires lipoic acid (101), which is involved in electron transport but does not interact with the substrate. The LDHs of *B. rettgeri* are extremely labile and lose activity when stored at −20°C; they are not stabilized by reducing agents. Partially purified DL-iLDH had a specific activity of about 100. Evidence that the enzyme molecule contained flavine was inconclusive; $(NH_4)_2SO_4$ was stimulatory, and although other salts were slightly stimulatory, only $MgSO_4$ was as effective as the ammonium salt. Separation of D(−)- and L(+)-LDH's was not achieved, and the preparation showed five times more activity with the D(−)-lactate, but because the rate of oxidation is additive when L(+)-lactate is added to D(−)-lactate, it is possible that there are two separate LDHs. Furthermore, the effects of heat on the two activities were different. *B. rettgeri* is now called *Eubacterium limnosum* (7).

P. pentosaceum (66) has a D(−)-iLDH which was also activated by NH_4^+; it was not affected by Na^+ at the same ionic strength. Unlike the LDH of *B. rettgeri*, the LDH of *P. pentosaceum* was fairly stable at 4°C and pH 5.3 to 5.4. The partially purified enzyme had a low specific activity (i.e., 5.7) and oxidized D(−)-lactate more readily then L(+)-lactate; these activities were not separated. Inhibitors included both thiol-binding compounds and flavoprotein inhibitors. Other properties of these enzymes are shown in Tables 11 through 14.

iLDH's of the lactic acid bacteria. In the lactic acid bacteria the iLDH's have an optimum pH on the acid side of neutral. Although these enzymes are known to be present in a variety of species, there has not been any systematic study of their distribution or of their properties. In *L. casei* the iLDH's were purified and studied (65) long before the main lactate-forming enzyme was detected. The L(+)- and D(−)-iLDH's in *L. plantarum* have also been characterized (82, 83). There has been a more complex study of the iLDH's of these two species than of the iLDH's of species in other genera which can use lactate, with the exception of the D(−)-iLDH of *E. coli*.

The specific activity of the L(+)-iLDH of *L. casei* was 9.2 (19% recovery), and that of the D(−)-iLDH was 1.82 (4.5% recovery). These low specific activities may be due to the use of an artificial electron acceptor. Although with other species it has not been certain whether there are separate D(−)- and L(+)-iLDH's, the two enzymes in *L. casei* can be clearly separated by electrophoresis (Garvie, unpublished data), using the method described for the iLDH's of leuconostocs (27). The L(+)-iLDH gave a stronger reaction than the D(−)-iLDH and had a slower migration rate. Both enzymes were slow moving under the conditions used. The L(+)-iLDH, but not the D(−)-iLDH, was able to reduce pyruvate to lactate with reduced methyl viologen as electron donor (65). The D(−)- and L(+)-iLDH's of *L. plantarum* can also be clearly separated by electrophoresis (83). The specific activities of these enzymes were also low, 5.74 (18% recovery) for the L(+)-iLDH and 30 (7% recovery) for the D(−)-iLDH. The D(−)-iLDH lost activity much more rapidly than the L(+)-iLDH, which was fairly stable. The molecular weight of the L(+)-iLDH was only 10,000, which is lower than the molecular weight estimated for any other LDH. The prosthetic group in the L(+)-iLDH is flavin mononucleotide, and that in the D(−)-iLDH is probably also a flavoprotein; thus, the iLDH's of the lactic acid bacteria resemble those of other types of bacteria. Where prosthetic groups have been examined it has been found that they may be either flavin adenine dinucleotide or flavin mononucleotide and that it appears to be tightly bound. Its presence has been difficult to demonstrate in several instances (12, 65, 66, 83, 104). However, most of the enzymes are inhibited by atabrine, which suggests a flavin enzyme. Only a few are inhibited by thiol-binding compounds (Table 15).

General observations. Like the nLDH's the iLDH's are not affected by the method of breaking the cells. A variety of techniques have been used, and none has been found to affect the LDHs adversely.

The importance of iLDH's to the bacteria and to the removal of lactate in the environment cannot be assessed until the distributions, functions, and properties of the enzymes are better known. Knowledge at present is fragmentary. *Propionibacterium* is one of the main genera able to utilize lactate (84), and the species of this genus are found in a lactate-rich environment (cheese); however, only one strain has been examined for its lactate-utilizing enzymes.

Clostridium is another genus which contains species able to use lactate (84), but I have found no work on *Clostridium* LDHs. Other bacteria

use lactate but may not have iLDH's, for *Peptostreptococcus elsdenii* (now called *Megasphaera elsdenii* [7]) converts lactate to a mixture of fatty acids.

CONTROL OF LACTATE PRODUCTION IN GROWING BACTERIAL CULTURES

The terms homofermentative (producing over 85% lactate) and heterofermentative (mixed end products) apply only to the lactic acid bacteria when they are growing with an adequate supply of glucose on a medium not limiting in other ways. When other carbohydrates, including those more reduced than glucose, are used, the end products of fermentation may change. van der Hamer (92) showed that *L. casei* formed L(+)-lactate and acetic acid from ribose when the carbohydrate supply was high, but D(−)-lactate and acetate when the supply was low or if the cells were metabolizing slowly because they were not adapted to growth on ribose. Ribose is metabolized mainly by the phosphoketolase system, and only a small amount of FDP is formed. When the FDP concentration in cells is low, the FDP-activated L(+)-nLDH has low activity. In the case of *L. casei*, the D(−)-nLDH converts pyruvate to D(−)-lactate. However, because the amount of this LDH is low (85), large amounts of lactate are formed, and some of the pyruvate is probably converted to acetate. Further studies (17) showed that even though lactate production was suppressed because glucose in the medium was limiting, the cells contained large amounts of L(+)-nLDH.

In the streptococci, a similar pattern of fermentation has been shown in *S. mutans* JC2 (107). When glucose was limiting, this strain formed some L(+)-lactate [there was no D(−)-nLDH] and also acetate, formate, and ethanol. The concentration of FDP in the cells was low, but the amount of LDH per milligram of cell protein was the same as in cells growing with adequate glucose. In another strain of *S. mutans*, in which there was no FDP control over the LDH, and in *S. bovis* ATCC 9809, lactate was formed when glucose was available both in excess and in limiting amounts, and the amount of LDH per cell was the same under both conditions. The L(+)-nLDH of *S. bovis* ATCC 9809 is under FDP control, and no satisfactory explanation has been proposed for the failure to form end products other than lactate. It is possible, however, that ATCC 9809 does not have the enzymes to form end products other than lactate.

Recent work in which *S. lactis* was used has demonstrated similar metabolic behavior. In this work altered end products were obtained by changing the available carbohydrate. Acetate and formate were formed when the cultures were growing on ribose. Strains which hydrolyzed lactose by β-galactosidase (EC 3.2.1.23) formed mixed end products, but strains with only β-phosphogalactosidase (EC 3.2.1.-) converted lactose to lactate. The change of end products occurred when FDP was in low supply, either because the metabolic rate was slow or because the pathway used lowered the FDP concentration. Two strains of *S. cremoris* formed only lactate from all carbohydrates, and it appears that these strains are unable to form acetate and formate. They were apparently unable to obtain enough energy from ribose and failed to grow on ribose-containing media (Garvie, unpublished data). Wild strains of *S. lactis* with low levels of lactose-hydrolyzing enzyme grow poorly on lactose, but the amount of LDH per milligram of cell protein is little different from the amount found in cheese starter strains with high levels of β-phosphogalactosidase.

The activities of FDP-activated nLDH's are affected by phosphate, and it has been suggested that the phosphate content of bacterial cells may influence the FDP-activated L(+)-nLDH's (10); however, the effect of phosphate has not yet been demonstrated in vivo. Phosphate is known to accumulate in the cells of *S. faecalis* and so might well influence the activity of the FDP-activated nLDH's. The FDP concentration in cells of *S. lactis* (and other streptococci) during logarithmic growth on mono- or disaccharides is about 15 mM. This level would fully activate the LDH at the concentration of phosphate likely to accumulate in a cell (10).

Other evidence that FDP affects the activities of LDHs in vivo has been obtained from *Bifidobacterium bifidum* (16). This species has an FDP-activated nLDH but lacks FDP aldolase. Fructose 6-phosphate is converted to acetyl phosphate and erythrose 4-phosphate. However, the cells have a low concentration of phosphofructokinase, which forms some FDP, and this in turn activates the nLDH. Bifidobacteria are separated from the homofermentative lactobacilli because they form acetate and some lactate from glucose but no CO_2 or ethanol (7).

The change from anaerobic to aerobic conditions can have a marked effect on metabolism. *Streptococcus faecium* grown aerobically had an L(+)-iLDH, particularly if ribose, glycerol, or pyruvate was the available carbohydrate (60). The Embden-Myerhof pathway would not operate under these conditions but would operate when fructose was available. The L(+)-iLDH was not found in fructose-grown cells. When the iLDH was present, the cells were able to grow

by using lactate as an energy source. Under anaerobic conditions there was no evidence that the lactate formed was subsequently metabolized. iLDH's have been found in many lactic acid bacteria grown in batch cultures on glucose, so that aeration and nonuse of the Embden-Myerhof pathway are not necessary for their formation. There is no evidence that any of these bacteria with iLDH's can grow by using lactate; indeed, most general bacteriological media contain considerable amounts of lactate (26), but lactic acid bacteria grow poorly in them in the absence of added carbohydrate. These nLDH's which can use lactate in vitro do so at an alkaline pH, and it is probable that the internal pH's of lactic acid bacteria are acid. If the iLDH's function in vivo, they would convert lactate to pyruvate, but pyruvate is not a suitable substrate for growth.

In bacteria other than the lactic acid bacteria, there is a different type of control over lactate production and utilization. The iLDH's are more important, and lactate is often only one of several end products of fermentation. The D(−)-nLDH of *E. coli* has a high K_m for pyruvate (5.0 mM at pH 6.4, rising to 7.2 mM at pH 7.5), and it has been suggested that this enzyme is inactive at low levels of pyruvate (91). *E. coli* grows well at pH 7.0 on media without added sugar, and since D(−)-lactate is involved in electron transport, the lactate is presumably formed from pyruvate by the D(−)-nLDH even when pyruvate is low. In *A. aerogenes* it has been suggested that an equilibrium exists between the two types of LDH present in the cells (68). Aerobic growth on glucose increased the amount of the nLDH in the cells compared with anaerobic growth, whereas lactate in the growth medium reduced the amount of nLDH (72).

The conditions of growth for inducing the production of D(−)- and L(+)-iLDH's in the *Enterobacteriaceae* are described above (iLDH's of *Escherichia coli* and iLDH's of other *Enterobacteriaceae*. The D(−)-iLDH in the *Enterobacteriaceae* is a constitutive enzyme, but it has been found that *A. aerogenes*, when grown in a medium containing both glucose and lactate, uses lactate only after the glucose has been exhausted (71). When *B. subtilis* is grown aerated on glucose, organic acids, including pyruvate, accumulate in the medium, but as the supply of glucose is exhausted, nLDH is formed and pyruvate is used (109). However, pyruvate is not the inducer of the LDH. In the absence of aeration, *B. subtilis* grows poorly, but the amount of nLDH per cell is much greater than when cells are grown aerobically.

Preferential use of carbon sources has also been demonstrated in anaerobic bacteria. *S. ruminantium* (75) formed L(+)-lactate from glucose and in batch culture used lactate when glucose was exhausted. However, no iLDH was detected. If this is a genuine example of an nLDH using lactate in vivo, it would be interesting, but more evidence will be required before any conclusion can be drawn. Other strains which are given the same species name behave differently and are unable to use lactate (75, 96).

The iLDH's in *B. rettgeri* are induced by growth on lactate (103), and such a culture only ferments lactate when lipoic acid is available to transport electrons released by the fermentation of lactate (101). The ability to ferment lactate is repressed when cells are grown on glucose, fructose, or pyruvate, and although lactate is formed, the cells are unable to use it. These cells have an increased level of nLDH compared with the level in lactate-grown cells (103). As with other species, *B. rettgeri* uses glucose preferentially when both glucose and lactate are available, and diauxic growth occurs in media with mixed substrates if glucose is limiting.

nLDH's can also be influenced by the nutrients available. *S. aureus* grown anaerobically with glucose forms lactate, and cells have a high level of L(+)-nLDH (24). The level increases when pyruvate is added to the medium. Aerobically grown cells form end products other than lactate and have a low level of nLDH. *S. epidermidis* has an FDP-activated L(+)-nLDH, and in this species the level of FDP in the cells controls lactate production (43).

Therefore there is evidence of control of nLDH's in vivo by both FDP and pyruvate, but I have found only one suggestion of control by ATP (38). The conditions under which an nLDH is functioning in a cell can only be guessed, and no strong evidence is available to show that either NAD or ATP affects the formation of lactate in a growing culture.

In growing cells lactate is the end product, and it is unlikely that nLDH's can convert it back to pyruvate. A number of properties of the enzymes indicate that this is so. (i) Many LDHs have high K_m values for lactate or are irreversible. (ii) The oxidation of lactate occurs at nonphysiological alkaline pH values and does not take place at low pH values. After electrophoresis the nLDH's of the lactobacilli cannot be detected at pH 5.5 (lactate as substrate) (34), but the pH in the cells may be near this value (39). (iii) Enzymes not reversible with NAD may be reversible with NAD analogs (70, 72, 98), but these substances are artificial. Only relatively few conditions and species have been examined, but it is evident that the production and utili-

zation of lactate by bacteria are controlled by a variety of factors which can operate differently in different species.

LDHs IN BACTERIAL TAXONOMY

Before LDHs were understood, lactate itself was used in separating species, particularly in the lactic acid bacteria. Enzymic techniques are now the preferred methods for determining the two isomers of lactate. The estimation of L(+)-lactate by using NAD and mammalian LDH is today routine, but the estimation of D(−)-lactate is less straightforward. A manometric method in which acetone-treated cells of *E. coli* strain B are used has been developed (93). In the original method lactate-grown cells were used. These contained both D(−)- and L(+)-iLDH's, but the latter enzyme should have been destroyed by the acetone treatment (44). The technique was not always satisfactory, and in some laboratories the L(+)-iLDH, which was still present in acetone-treated lactate-grown cells, interfered with the estimation of D(−)-lactate. Glucose-grown cells, which lacked L(+)-iLDH, were more satisfactory (25). The uptake of O_2 during lactate utilization is not as sensitive a method as the enzymic techniques in which nLDH's are used. Unfortunately, D(−)-nLDH's must be prepared from bacteria commercially or in a research laboratory, and these enzymes have a higher K_m for lactate than mammalian L(+)-nLDH (Table 5). The estimation of D(−)-lactate is less satisfactory than the estimation of L(+)-lactate. Even so, enzymic methods (69, 85) are preferable to the original technique of ether extraction and determination of the optical rotation of the salt formed.

Differences in LDHs, particularly nLDH's, have shown that, at least in the lactic acid bacteria, the D(−)-, L(+)-, and DL-lactate formers can be further divided. The first studies used electrophoresis of LDHs in crude cell extracts of lactobacilli (34) and leuconostocs (27). The emphasis was on the nLDH's, and distinct patterns were found to be associated with species of *Lactobacillus*, although more than one pattern was found in *L. acidophilus* and *Lactobacillus brevis* (34). In the leuconostocs the reverse was the case, and five species had electrophoretically identical D(−)-LDHs; only the acidophilic *L. oenos* was distinct. Later work (78) showed that the D(−)-nLDH's of *L. confusus* and *L. viridescens* were not separated from those of the nonacidophilic leuconostocs. The iLDH's were included in both studies; in the lactobacilli only those enzymes showing with phenazinemethosulfate and Nitro Blue Tetrazolium were observed (34). In the leuconostocs the iLDH's were identified separately from the nLDH's by using dichlorophenol indophenol as the hydrogen acceptor, but the enzymes were too weak to be used in classification studies.

The study of the LDHs of the leuconostocs and some lactobacilli was extended by using immunological techniques, and relationships were shown between the D(−)-nLDH's of the leuconostocs and lactobacilli but not with the D(−)-nLDH's of *B. rettgeri* and the pediococci (36). The serological work confirmed the similarity between the LDHs of the leuconostocs and *L. viridescens*. The D(−)-nLDH's of the leuconostocs could be separated into several groups by immunological techniques (50), and the same groupings of strains and species were found by deoxyribonucleic acid-deoxyribonucleic acid hybridization studies (29, 51).

In this immunological work the D(−)-nLDH of *L. oenos* was found to be different from the D(−)-nLDH's of other species. A relationship between the nLDH of *L. oenos* and the nLDH's of other leuconostocs was demonstrated because hybrid enzymes were formed between the nLDH of *L. oenos* and the nLDH's of other *Leuconostoc* species and of *L. viridescens* and *L. confusus* but not with the D(−)-nLDH's of other lactobacilli (28). Immunological studies in which *L. casei* L(+)-nLDH was used showed a cross-reaction only with *L. plantarum* (an nLDH unaffected by FDP) and not with any group D or group N streptococci which have FDP-activated nLDH's (40).

Studies of LDHs in these genera have confirmed species which had been proposed earlier (7) and even led to the discovery of a new species, *Lactobacillus jensenii* (37). The similarities between the LDHs of *L. fermentum* and *L. cellobiosus* indicate that these may not be separate species (7, 34, 78).

In the streptococci, electrophoresis of LDHs has been used (30, 31, 32, 33), but as gels have to be developed with pyruvate, the technique is less satisfactory than when it is applied to the LDHs of other genera. The streptococcal nLDH's can be used in the separation of species by using the influence of pyruvate, FDP, and phosphate concentration on the activities of the enzymes at pH 7.0 and 5.5 (30, 31, 32, 33). The study of streptococcal LDHs has confirmed one new species, *Streptococcus raffinolactis*, and casts doubt on the species rank of *S. cremoris* (30).

Recently, different electrophoretic patterns have been shown to relate to different species of the genus *Pediococcus* (3). Most species form DL-lactate and have both D(−)- and L(+)-nLDH's; however, *Pediococcus dextrinicus*

forms only L(+)-lactate and has an FDP-dependent nLDH, whereas in *Pediococcus halophilus* the L(+)-nLDH is not under FDP control, although this species also forms only L(+)-lactate.

In other bacteria LDH studies have not been used in classification to any extent. The LDH electrophoresis patterns were studied in some *S. aureus* strains, and multiple LDHs appeared to correlate with high coagulase levels (86). The results were not conclusive. The classification of the gram-positive, catalase-positive cocci has long been troublesome. Recently, preliminary work has shown that the type of lactate formed and the LDHs in the cells may assist in the separation of species among the staphylococci (42).

Lactate production and utilization and the LDHs found in the strains studied suggest that strains called *S. ruminantium* may not all belong to the same species because some strains form D(−)-lactate, some form L(+)-lactate, some can use lactate, and others cannot use lactate (75, 96).

Evolution in bacteria is only beginning to be understood. Relationships between species are being sought in many genera by using deoxyribonucleic acid-deoxyribonucleic acid hybridization and, less often, ribonucleic acid-deoxyribonucleic acid hybridization studies. The results obtained by the techniques in use are not fully understood. It is possible that more information of greater reliability would be obtained from a study of proteins. A start has been made with aldolases (61), which are widely distributed, and LDHs might also prove to be valuable. For instance what is the significance or advantage of forming D(−)- or L(+)-lactate? At what stage was there a separation into the two different LDHs, or did DL-lactate formers come first? The FDP-controlled nLDH's are also interesting. The distribution of species with this type of LDH is erratic. Has this type of LDH developed only once, and therefore do all species with an FDP-activated LDH have a common ancestor, or has FDP control arisen on several occasions? If FDP control has developed separately in different genera, it would seem to give the organisms an advantage. Why has an FDP-activated nLDH persisted in the bifidobacteria when they have lost their FDP aldolase?

CONCLUSIONS

From the evidence presented above, it can be seen that bacterial LDHs, both nLDH's and iLDH's, are very varied in every property. Knowledge of these enzymes is, in many instances, patchy; a particular enzyme has been studied, often in some depth, but when the investigator has moved elsewhere or to other topics, interest has waned. Few enzymes have been studied in more than one laboratory, and even the D(−)-iLDH of *E. coli* has been examined only as part of a larger study on electron transport.

It is hoped that workers with the ability to unravel the complexities of enzymes will realize that bacteria are a rich field for investigation and that more bacterial LDHs will be examined in depth.

In the eucaryotes it is reasonable to call an enzyme which catalyzes the reaction pyruvate ⇌ lactate an LDH; however, in the procaryotes pyruvate reductase would be a better name for the nLDH's, for this is their function, and LDH should be reserved for the iLDH's, for this is their function. This nomenclature would be against the recommendations of the Enzyme Commission but has already been used in some publications (68, 78).

LITERATURE CITED

1. **Alizade, M. A., and H. Simon.** 1973. Zum Mechanismus und zur Kompartmentierung der L- und D-Lactat-Bildung aus L-Malat bzw D-Glucose in *Leuconostoc mesenteroides*. Z. Physiol. Chem. **354**:163–168.
2. **Anders, R. F., H. A. Jonas, and G. R. Jago.** 1970. A survey of the lactate dehydrogenase activities in group N streptococci. Aust. J. Dairy Technol. **25**:73–76.
3. **Back, W.** 1978. Zur Taxonomie der Gattung Pediococcus. Phänotypische und genotypische Abgrenzung der bisher bekannten Arten sowie Beschreibung einer neuen bierschadlichen Art: *Pediococcus inopinatus*. Sonderd. Brauwiss. **9**: 237–250.
4. **Barman, T. E.** 1969. Enzyme handbook, vol. 1. Springer Verlag, Berlin.
5. **Brown, A. T., C. P. Christian, and R. L. Eilert.** 1975. Purification, characterization and regulation of a nicotinamide adenine dinucleotide-dependent lactate dehydrogenase from *Actinomyces viscosus*. J. Bacteriol. **122**:1126–1135.
6. **Brown, A. T., and C. L. Wittenberger.** 1972. Fructose 1,6-diphosphate-dependent lactate dehydrogenase from a cariogenic streptococcus: purification and regulatory properties. J. Bacteriol. **110**:604–615.
7. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
8. **Carr, J. G., C. V. Cutting, and G. C. Whiting (ed.).** 1975. Lactic-acid bacteria in beverages and foods. Academic Press Inc., London.
9. **Christensen, W. B., M. J. Johnson, and W. H. Peterson.** 1939. Properties of the lactic-acid racemizing enzyme of *Clostridium butylicum*. J. Biol. Chem. **127**:421–430.

10. **Crow, V. L., and G. C. Pritchard.** 1977. Fructose 1,6-diphosphate-activated L-lactate dehydrogenase from *Streptococcus lactis*: kinetic properties and factors affecting activation. J. Bacteriol. **131**:82-91.
11. **deLey, J.** 1964. Comparative biochemistry and enzymology in bacterial classification, p. 164-195. *In* G. C. Ainsworth and P. H. A. Sneath (ed.), Microbial classification, 12th Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge, England.
12. **deLey, J., and J. Schel.** 1959. Studies on the metabolism of *Acetobacter peroxydans*. II. The enzymic mechanism of lactate metabolism. Biochim. Biophys. Acta **35**:154-165.
13. **Dennis, D., and N. O. Kaplan.** 1960. D- and L-Lactic acid dehydrogenases in *Lactobacillus plantarum*. J. Biol. Chem. **235**:810-818.
14. **Dennis, D., and N. O. Kaplan.** 1963. Lactic-acid racemization in *Clostridium butylicum*. Biochem. Z. **338**:485-495.
15. **Dennis, D., M. Reichlung, and N. O. Kaplan.** 1965. Lactic-acid racemization. Ann. N. Y. Acad. Sci. **119**:868-876.
16. **de Vries, W., S. J. Gerbrandy, and A. H. Stouthamer.** 1967. Carbohydrate metabolism in *Bifidobacterium bifidum*. Biochim. Biophys. Acta **136**:415-425.
17. **de Vries, W., W. M. C. Kapteijn, E. G. van der Beek, and A. H. Stouthamer.** 1970. Molar growth yields and fermentation balances of *Lactobacillus casei* L3 in batch cultures and continuous cultures. J. Gen. Microbiol. **63**:333-345.
18. **Doelle, H. W.** 1971. Nicotinamide adenine dinucleotide-dependent and nicotinamide adenine dinucleotide-independent lactate dehydrogenases in homofermentative and heterofermentative lactic acid bacteria. J. Bacteriol. **108**:1284-1289.
19. **Doelle, H. W.** 1971. Influence of carboxylic acids on the stereospecific nicotinamide adenine dinucleotide-dependent and nicotinamide adenine dinucleotide-independent lactate dehydrogenases of *Leuconostoc mesenteroides*. J. Bacteriol. **108**:1290-1295.
20. **Dynon, M. K., G. R. Jago, and B. E. Davidson.** 1972. The sub-unit structure of lactate dehydrogenase from *Streptococcus cremoris* US3. Eur. J. Biochem. **30**:348-353.
21. **Eisenberg, R. J., M. Elchisak, and J. Rudd.** 1976. Regulation of lactate dehydrogenase activity in *Rothia dentocariosa* by fructose 1,6-diphosphate and adenosine 5-triphosphate. J. Bacteriol. **126**:1344-1346.
22. **Futai, M.** 1973. Membrane D-lactate dehydrogenase from *Escherichia coli*. Purification and properties. Biochemistry **12**:2468-2474.
23. **Garland, R. C.** 1973. Purification and properties of a DL-lactate dehydrogenase from *Leuconostoc mesenteroides*. Arch. Biochem. Biophys. **157**:36-43.
24. **Garrard, W., and J. Lascelles.** 1968. Regulation of *Staphylococcus aureus* lactate dehydrogenase. J. Bacteriol. **95**:152-156.
25. **Garvie, E. I.** 1966. A note on the estimation of D(−)-lactic acid using lactic dehydrogenase. J. Dairy Res. **33**:65-66.
26. **Garvie, E. I.** 1967. The production of L(+)- and D(−)-lactic acid in cultures of some lactic acid bacteria, with a special study of *Lactobacillus acidophilus* NCDO 2. J. Dairy Res. **34**:31-38.
27. **Garvie, E. I.** 1969. Lactic dehydrogenases of strains of the genus *Leuconostoc*. J. Gen. Microbiol. **58**:85-94.
28. **Garvie, E. I.** 1975. Some properties of gas-forming lactic-acid bacteria and their significance in classification, p. 339-349. *In* J. G. Carr, C. V. Cutting, and G. C. Whiting (ed.), The lactic acid bacteria in beverages and food. Academic Press Inc., London.
29. **Garvie, E. I.** 1976. Hybridization between the deoxyribonucleic acids of some strains of heterofermentative lactic acid bacteria. Int. J. Syst. Bacteriol. **26**:116-122.
30. **Garvie, E. I.** 1978. *Streptococcus raffinolactis* Orla-Jensen and Hansen, a group N streptococcus found in raw milk. Int. J. Syst. Bacteriol. **28**:190-193.
31. **Garvie, E. I.** 1978. Lactate dehydrogenases of *Streptococcus thermophilus*. J. Dairy Res. **45**: 515-518.
32. **Garvie, E. I., and A. J. Bramley.** 1979. *Streptococcus uberis*: an approach to its classification. J. Appl. Bacteriol. **46**:295-304.
33. **Garvie, E. I., and A. J. Bramley.** 1979. *Streptococcus bovis*: an approach to its classification and its importance as a cause of bovine mastitis. J. Appl. Bacteriol. **46**:557-566.
34. **Gasser, F.** 1970. Electrophoretic characterisation of lactic dehydrogenase in the genus *Lactobacillus*. J. Gen. Microbiol. **62**:223-239.
35. **Gasser, F., M. Doudoroff, and R. Contopoulos.** 1970. Purification and properties of NAD dependent lactic dehydrogenases of different species of *Lactobacillus*. J. Gen. Microbiol. **62**: 241-250.
36. **Gasser, F., and C. Gasser.** 1971. Immunological relationships among lactic dehydrogenases in the genera *Lactobacillus* and *Leuconostoc*. J. Bacteriol. **106**:113-125.
37. **Gasser, F., M. Mandel, and M. Rogosa.** 1970. *Lactobacillus jensenii* sp. nov., a new representative of the subgenus *Thermobacterium*. J. Gen. Microbiol. **62**:219-222.
38. **Gordon, G. L., and H. W. Doelle.** 1974. Molecular aspects for the metabolic regulation of the nicotinamide adenine dinuclotide-dependent D(−)-lactate dehydrogenase from *Leuconostoc*. Microbios **9**:199-215.
39. **Gordon, G. L., and H. W. Doelle.** 1975. Production of racemic lactic acid in *Pediococcus cerevisiae* cultures by two lactate dehydrogenases. J. Bacteriol. **121**:600-607.
40. **Gordon, G. L., and H. W. Doelle.** 1976. Purification, properties and immunological relationship of L(+)-lactate dehydrogenase from *Lactobacillus casei*. Eur. J. Biochem. **67**:543-555.
41. **Gotz, F., and K. H. Schleifer.** 1975. Purification and properties of a fructose 1,6-diphosphate activated L-lactate dehydrogenase from *Staphylococcus epidermidis*. Arch. Microbiol. **105**:

303-312.
42. **Gotz, F., and K. H. Schleifer.** 1976. Comparative biochemistry of lactate dehydrogenases from staphylococci. Zentralbl. Bakteriol. Parasitenkol. Infektionskr. Abt. 1 Suppl. **5**:245-252.
43. **Gotz, F., and K. H. Schleifer.** 1978. Biochemical properties and the physiological role of the fructose 1,6-biphosphate activated L-lactate dehydrogenase from *Staphylococcus epidermidis*. Eur. J. Biochem. **90**:555-561.
44. **Haugaard, N.** 1959. D- and L-Lactic acid oxidases of *Escherichia coli*. Biochim. Biophys. Acta **31**:66-72.
45. **Hensel, R., U. Mayr, H. Fujiki, and O. Kandler.** 1977. Comparative studies of lactate dehydrogenases in lactic-acid bacteria. Amino acid composition of an active site region and chemical properties of the L-lactate dehydrogenase of *Lactobacillus casei, Lactobacillus curvatus, Lactobacillus plantarum* and *Lactobacillus acidophilus*. Eur. J. Biochem. **80**: 83-92.
46. **Hensel, R., U. Mayr, K. O. Stetter, and O. Kandler.** 1977. Comparative studies of lactic-acid dehydrogenases in lactic acid bacteria. I. Purification and kinetics of the allosteric L-lactic acid dehydrogenase from *Lactobacillus casei* ssp. *casei* and *Lactobacillus curvatus*. Arch. Microbiol. **112**:81-93.
47. **Hiyama, T., S. Fukui, and K. Kitahara.** 1968. Purification and properties of lactate racemase from *Lactobacillus sake*. J. Biochem. (Tokyo) **64**:99-107.
48. **Holland, R., and G. C. Pritchard.** 1975. Regulation of the L-lactate dehydrogenase from *Lactobacillus casei* by fructose 1,6-diphosphate and metal ions. J. Bacteriol. **121**:777-784.
49. **Hontebeyrie, M., and F. Gasser.** 1973. Separation et purification de la D- lactico-déshydrogenase et de la glucose-6-phosphate déshydrogenase de *Leuconostoc lactis*. Etude de quelques proprietés. Biochemie **55**:1047-1056.
50. **Hontebeyrie, M., and F. Gasser.** 1975. Comparative immunological relationships of two distinct sets of isofunctional dehydrogenases in the genus *Leuconostoc*. Int. J. Syst. Bacteriol. **25**:1-6.
51. **Hontebeyrie, M., and F. Gasser.** 1977. Deoxyribonucleic acid homologies in the genus *Leuconostoc*. Int. J. Syst. Bacteriol. **27**:9-14.
52. **Jago, G. R., L. W. Nichol, K. O'Dea, and W. H. Sawyer.** 1971. Physicochemical studies on the lactate dehydrogenase of *Streptococcus cremoris* US_3. The effect of modifiers. Biochim. Biophys. Acta **250**:271-285.
53. **Jonas, H. A., R. F. Anders, and G. R. Jago.** 1972. Factors affecting the activity of the lactate dehydrogenase of *Streptococcus cremoris*. J. Bacteriol. **111**:397-403.
54. **Katagiri, H., and K. Kitahara.** 1937. Racemiase, an enzyme which catalyses racemation of lactic acids. Biochem. J. **31**:909-914.
55. **Kaufmann, E., and S. Dikstein.** 1961. A D-lactic dehydrogenase from *Leuconostoc mesenteroides*. Nature (London) **190**:346.
56. **Kelly, N., M. Delaney, and P. O'Cara.** 1978. Affinity chromatography of bacterial lactate dehydrogenases. Biochem. J. **171**:543-547.
57. **Kemp, M. B.** 1972. D- and L-Lactate dehydrogenases of *Pseudomonas aeruginosa*. Biochem. J. **130**:307-309.
58. **Kline, E. S., and H. R. Mahler.** 1965. The lactic dehydrogenases of *E. coli*. Ann. N. Y. Acad. Sci. **119**:905-917.
59. **Kohn, L. D., and H. R. Kaback.** 1973. Mechanism of active transport in isolated membrane vesicles. XV. Purification and properties of the membrane-bound D-lactate dehydrogenase from *Escherichia coli*. J. Biol. Chem. **248**: 7012-7017.
60. **London, J.** 1968. Regulation and function of lactate oxidation in *Streptococcus faecium*. J. Bacteriol. **95**:1380-1387.
61. **London, J., and K. Kline.** 1973. Aldolase of lactic acid bacteria: a case history in the use of an enzyme as an evolutionary marker. Bacteriol. Rev. **37**:453-478.
62. **Manderson, G. J., and H. W. Doelle.** 1972. The effect of oxygen and pH on glucose metabolism of *Lactobacillus casei* var. *rhamnosus* ATCC 7469. Antonie van Leeuwenhoek J. Microbiol. Serol. **38**:223-240.
63. **Mickelson, M. N.** 1967. Aerobic metabolism of *Streptococcus agalactiae*. J. Bacteriol. **94**:184-191.
64. **Mizushima, S., T. Hiyama, and K. Kitahara.** 1964. Quantitative studies on glycolytic enzymes in *Lactobacillus planatarum*. III. Intracellular activities of reverse reaction of D- and L-lactate dehydrogenases during glucose fermentation. J. Gen. Appl. Microbiol. **10**:33-44.
65. **Mizushima, S., and K. Kitahara.** 1962. Purification and properties of lactic dehydrogenase of *Lactobacillus casei*. J. Gen. Appl. Microbiol. **8**:130-141.
66. **Molinari, R., and F. J. S. Lara.** 1960. The lactic dehydrogenase of *Propionibacterium pentosaceum*. Biochem J. **75**:57-65.
67. **Mou, L., D. P. Mulvena., H. A. Jonas, and G. R. Jago.** 1972. Purification and properties of nicotinamide adenine dinucleotide-dependent D- and L-lactate dehydrogenases in group N *Streptococcus*. J. Bacteriol. **111**:392-396.
68. **Neijssel, O. M., T. O. Sutherland-Miller, and D. W. Tempest.** 1978. Pyruvate reductase and D-lactate dehydrogenase: a possible mechanism for avoiding energy conservation at site 1 of the respiratory chain in *Klebsiella aerogenes* NCTC 418. Proc. Soc. Gen. Microbiol. **5**: 49.
69. **Neimark, H., and R. M. Lemcke.** 1972. Occurrence and properties of lactic dehydrogenases of fermentative mycoplasmas. J. Bacteriol. **111**:633-640.
70. **Neimark, H., and M. C. Tung.** 1973. Properties of a fructose 1,6-diphosphate-activated lactate dehydrogenase from *Acholeplasma laidlawii* type A. J. Bacteriol. **114**:1025-1033.
71. **Pascal, M.-C., and F. Pichinoty.** 1965. Regulation de la biosynthése et de la fonction dés

D et L lactate déshydrogenase chez *Aerobacter aerogenes*. Biochim. Biophys. Acta **105**:54-69.

72. **Pascal, M.-C., and F. Pichinoty.** 1966. Contribution a l'étude de la pyruvate-reductase d' *Aerobacter aerogenes*. Bull. Soc. Chim. Biol. **48**:583-593.
73. **Pesce, A., R. H. McKay, F. Stolzenbach, R. D. Cahn, and N. O. Kaplan.** 1964. The comparative enzymology of lactic dehydrogenases. I. Properties of the crystalline beef and chicken enzymes. J. Biol. Chem. **239**:1753-1761.
74. **Sawula, R. V., and I. Suzuki.** 1970. The effect of enzyme concentration on the kinetics of D-lactate dehydrogenase from *Aerobacter aerogenes*. Biochim. Biophys. Res. Commun. **40**: 1096-1101.
75. **Scheifinger, C. C., M. J. Latham, and M. J. Wolin.** 1975. Relationship of lactate dehydrogenase specificity and growth rate to lactate metabolism by *Selenomonas ruminantium*. Appl. Microbiol. **30**:916-921.
76. **Schutz, M., and F. Radler.** 1973. Das Malatenzym von *Lactobacillus plantarum* und *Leuconostoc mesenteroides*. Arch. Mikrobiol. **91**: 183-202.
77. **Schwert, G. W., and A. D. Winer.** 1963. Lactate dehydrogenase, p. 127-148. *In* P. D. Boyer, H. Lardy, and K. Myrback (ed.), The enzymes, 2nd ed. Academic Press Inc., New York.
78. **Sharpe, M. E., E. I. Garvie, and R. H. Tilbury.** 1972. Some slime-forming heterofermentative species of the genus *Lactobacillus*. Appl. Microbiol. **23**:389-397.
79. **Sharpe, M. E., M. J. Latham, E. I. Garvie, J. Zirngibl, and O. Kandler.** 1973. Two new species of *Lactobacillus* isolated from the bovine rumen, *Lactobacillus ruminis* sp. nov and *Lactobacillus vitulinus* sp. nov. J. Gen. Microbiol. **77**:37-49.
80. **Short, S. A., and H. R. Kaback.** 1974. Mechanisms of active transport in isolated membrane vesicles. Further studies on amino acid transport in *Staphylococcus aureus* membrane vesicles. J. Biol. Chem. **249**:4275-4281.
81. **Smith, S. L., P. J. van Demark, and J. Fabricant.** 1963. Respiratory pathways in *Mycoplasma*. I. Lactate oxidation by *Mycoplasma gallisepticum*. J. Bacteriol. **86**:803-807.
82. **Snoswell, A. M.** 1959. Flavins of *Lactobacillus arabinosus* 17.5. A lactic dehydrogenase containing a flavin prosthetic group. Aust. J. Exp. Biol. Med. **37**:49-64.
83. **Snoswell, A. M.** 1963. Oxidized nicotinamide-adenine dinucleotide-independent lactate dehydrogenases of *Lactobacillus arabinosus* 17.5. Biochim. Biophys. Acta **77**:7-19.
84. **Sokatch, J. R.** 1969. Bacterial physiology and metabolism. Academic Press Inc., London.
85. **Stetter, K. O., and O. Kandler.** 1973. Untersuchungen zur Entstehung von D. L. Milchsaüre bei Lactobacillen und Charakterisierung einer Milchsaureracemase bei einigen Arten der nutergattung Streptobacterium. Arch. Microbiol. **94**:221-247.
86. **Stockland, A. E., and C. L. San Clemente.** 1968. Lactate dehydrogenase activity in certain strains of *Staphylococcus aureus*. J. Bacteriol. **95**:74-80.
87. **Stockland, A. E., and C. L. San Clemente.** 1969. Multiple forms of lactate dehydrogenases in *Staphylococcus aureus*. J. Bacteriol. **100**: 347-353.
88. **Szulmajster, J., M. Grunberg-Manago, and C. Delavier-Klutchko.** 1953. Properties d'une lactico-déshydrogenase isolée des "particules" de *E. coli*. Bull. Soc. Chim. Biol. **35**:1381-1393.
89. **Tanaka, Y., Y. Anraku, and M. Futai.** 1976. *Escherichia coli* membrane D-lactate dehydrogenase. Isolation of the enzyme in aggregated form and its activation by Triton X-100 and phospholipids. J. Biochem. (Tokyo) **80**:821-830.
90. **Tarmy, E. M., and N. O. Kaplan.** 1968. Chemical characterization of D-lactate dehydrogenase from *Escherichia coli* B. J. Biol. Chem. **243**:2579-2586.
91. **Tarmy, E. M., and N. O. Kaplan.** 1968. Kinetics of *Escherichia coli* B D-lactate dehydrogenase and evidence for pyruvate controlled change in conformation. J. Biol. Chem. **243**:2587-2596.
92. **van der Hamer, C. J. A.** 1960. The carbohydrate metabolism of the lactic acid bacteria. Proefschrift Rijksuniversiteit te Utrecht, p. 9-126.
93. **van der Hamer, C. J. A., and R. W. Elias.** 1958. A method for the estimation of D(−)-lactic acid. Biochim. Biophys. Acta **29**:556-562.
94. **van Gylswyk, N. O.** 1977. Activation of NAD dependent lactate dehydrogenase in *Butyrivibrio fibrisolvens* by fructose 1,6-diphosphate. J. Gen. Microbiol. **99**:441-443.
95. **Walker, H., and R. G. Eagon.** 1964. Lactic dehydrogenases of *Pseudomonas natriegens*. J. Bacteriol. **88**:25-30.
96. **Wallace, R. J.** 1978. Control of lactate production by *Selenomonas ruminantium*: homotropic activation of lactate dehydrogenase by pyruvate. J. Gen. Microbiol. **107**:45-52.
97. **Weerkamp, A., and R. D. MacElroy.** 1972. Lactate dehydrogenase from an extremely thermophilic bacillus. Arch. Microbiol. **85**:113-122.
98. **Wittenberger, C. L.** 1966. Unusual kinetic properties of a DPN-linked lactate dehydrogenase from *Butyribacterium rettgeri*. Biochem. Biophys. Res. Commun. **22**:729-736.
99. **Wittenberger, C. L.** 1968. Kinetic studies on the inhibition of a D(−) specific lactate dehydrogenase by adenosine triphosphate. J. Biol. Chem. **243**:3067-3075.
100. **Wittenberger, C. L., and N. Angelo.** 1970. Purification and properties of a fructose 1,6-diphosphate-activated lactate dehydrogenase from *Streptococcus faecalis*. J. Bacteriol. **101**: 717-724.
101. **Wittenberger, C. L., and M. Flavin.** 1963. *Butyribacterium rettgeri*: a role of lipoic acid in anaerobic electron transport. J. Biol. Chem. **238**:2529-2536.
102. **Wittenberger, C. L., and J. G. Fulco.** 1967.

Purification and allosteric properties of a nicotinamide adenine dinucleotide-linked D(−) specific lactate dehydrogenase from *Butyribacterium rettgeri*. J. Biol. Chem. **242**:2917–2924.

103. **Wittenberger, C. L., and A. S. Haaf.** 1964. Lactate-degrading system in *Butyribacterium rettgeri* subject to glucose repression. J. Bacteriol. **88**:896–903.
104. **Wittenberger, C. L., and A. S. Haaf.** 1966. Isolation of NAD-dependent and NAD-independent lactate dehydrogenases from extracts of *Butyribacterium rettgeri*. Biochim. Biophys. Acta **122**:393–405.
105. **Wittenberger, C. L., M. P. Palumbo, R. B. Bridges, and A. T. Brown.** 1971. Mechanisms for regulating the activity of constitutive glucose degradative pathways in *Streptococcus faecalis*. J. Dent. Res. **50**:1094–1103.
106. **Wolin, M. J.** 1964. Fructose 1,6-diphosphate requirement of streptococcal lactic dehydrogenases. Science **146**:775–777.
107. **Yamada, T., and J. Carlsson.** 1975. Regulation of lactate dehydrogenase and change of fermentation products in streptococci. J. Bacteriol. **124**:55–61.
108. **Yamada, T., K. Endo, and S. Araya.** 1976. A fructose 1,6-diphosphate independant L-lactate dehydrogenase in a strain of *Streptococcus mutans*. Arch. Oral Biol. **21**:233–236.
109. **Yashphe, J., J. A. Hoch, and N. O. Kaplan.** 1978. Regulation of lactate dehydrogenase synthesis in *Bacillus subtilis*. Biochim. Biophys. Acta **544**:1–7.
110. **Yoshida, A.** 1965. Enzymic properties of lactate dehydrogenase of *Bacillus subtilis*. Biochim. Biophys. Acta **99**:66–77.
111. **Yoshida, A., and E. Freese.** 1965. Purification and chemical characterization of lactate dehydrogenase of *Bacillus subtilis*. Biochim. Biophys. Acta **99**:56–65.